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PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

Studies in the Mechanism of Enzyme Action. I.—Rôle of the Reaction of the Medium in Fixing the Optimum Temperature of a Ferment.

By Arthur Compton, M.B., D.Sc., Mackinnon Student, British Medical Association Scholar.

(Communicated by Prof. B. Moore, F.R.S. Received July 24, 1920.)

Previously, it has been shown for the enzyme *maltase*—enzyme requiring an acid medium in which to act to best advantage—that increase in the acidity or hydrogen ion concentration of the medium in which the enzyme acts, beyond the optimum acidity, leads to a fall of optimum temperature.* The mechanism of this temperature effect appears clearly to be due to a certain disablement of the enzyme, brought about by the presence of excess of acid, for the fall of optimum temperature which occurs is rigorously proportional to the decrease of enzymic activity, estimated at the optimum temperature point; which decrease of activity is itself a function of the degree of acidity of the medium in excess of that necessary to produce optimum activation.* Being in this way a disablement effect, the question arises whether, by adding to the quantity of enzyme in action, the lowering of optimum temperature which takes place can be controlled. To answer that question, the experiments described in the present paper were undertaken.

For the investigation, the enzyme used is the *maltase* of *Aspergillus oryzæ*, the same preparation being employed as studied by us in two previous communications,† a specially active specimen of takadiastase, purified by repeated solution in water and reprecipitation by alcohol.

Fig. 1, based on one of the foregoing communications,* indicates the general lowering of the temperature range and corresponding fall of optimum temperature, for the *maltase* of the preparation in question, when

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* Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 88, pp. 408–417 (1915).

† Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 88, pp. 258–263 (1914), and *loc. cit.*

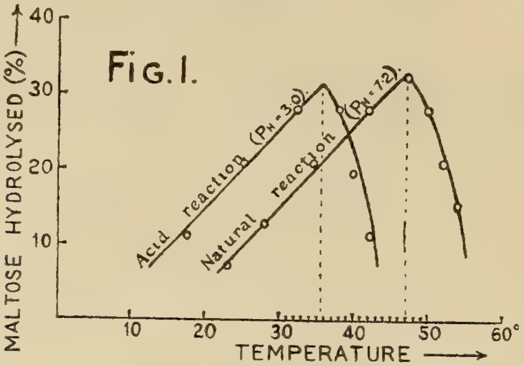

Fig. 1.

0·6 $cm.^3$ M/100 H^2SO^4 is added to a reaction mixture of total volume 5 $cm.^3$, containing in solution 3 mgrm. of the enzyme preparation (enzyme concentration, 6×10^{-4} mgrm. per $cm.^3$). This addition of acid, as indicated on the diagram, corresponds to a H^+ concentration of $10^{-3 \cdot 0}$, the H^+ concentration for the natural reaction (*i.e.*, without addition of acid) being $10^{-7 \cdot 2}$. Under these circumstances, the *maltase* of the preparation is seen to undergo a lowering of its optimum temperature from +47° (natural reaction) to +35·5° (above mentioned acid reaction).

In the experiments which follow, we have always operated in presence of this constant addition of acid: 0·6 $cm.^3$ H^2SO^4 per 5 $cm.^3$ of the reaction mixture, for disabling effect; while the quantities of enzyme present have varied from 3 mgrm. to 7 mgrm. per 5 $cm.^3$ total mixture, that is to say, enzyme concentrations varying between 6×10^{-4} and 14×10^{-4} grm. per $cm.^3$. The substrate concentration throughout was M/20, or 18×10^{-3} grm. of hydrated maltose per $cm.^3$ of the reaction mixture. It was not practical to study the course of events for superior enzyme concentrations, owing to complete hydrolysis of the substrate being attained, in the conditions of the experiments, and consequent want of definition in the matter of the optimum temperature point.

The practical details of the experiments carried out were as follows: 90 mgrm. of hydrated maltose, 0·6 $cm.^3$ M/100 H^2SO^4, and 3·4 $cm.^3$ of pure water—prepared from distilled water by redistillation under diminished pressure—were introduced into four series of eight dry, clean, "resistance glass" test-tubes, rendered clean by successive washing in acid, water, and steam. The tubes were then plunged into water thermostats, regulated to the 1/10th of a degree, and, an equilibrium of temperature having been established between their contents and the thermostats, into corresponding tubes of the respective series were introduced in portions of 1 cm.^{3}, solutions of the enzyme, prepared half-hour to one hour previously, containing respectively 3·0 mgrm., 4·75 mgrm., 6·0 mgrm., and 7·0 mgrm. of the enzyme powder per cm.^{3}. The tubes were now closed with selected corks—treated just before use with boiling water from the jet of a wash bottle—and incubated at the temperatures of the thermostats for 16 hours. At the end of this time, the enzyme action was stopped by plunging the tubes for seven minutes into boiling water, the corks having first been withdrawn, and each rapidly washed with 1 cm.^{3} of distilled water, the washings being added to the contents of the corresponding tubes. On being cooled to laboratory temperature, the contents of each tube were diluted to 50 cm.^{3}, and the percentage of maltose hydrolysed, determined by Bertrand's method,* on 20 cm.^{3} of the diluted mixture. The numbers obtained are set out in the accompanying Table.

Temperature at beginning and end of each experiment.	Maltose hydrolysed per cent. with the following enzyme concentrations.			
	6×10^{-4}.	$9{\cdot}5 \times 10^{-4}$.	12×10^{-4}.	14×10^{-4}.
17·0	—	—	21·1	28·2
17·0–17·1	—	16·9		
17·7–17·8	11·4			
22·9–23·2	—	—	—	46·4
24·7–24·9	—	—	40·6	
25·0–24·7	—	28·2		
25·2–25·4	21·1			
32·0	—	—	65·2	
32·1–32·0	—	—	—	82·0
32·2–32·0	—	47·9		
32·2–32·1	28·2			
35·4	—	—	—	94·3
35·5	—	52·5	75·1	
37·0–36·9	—	—	—	92·5
37·8–38·2	28·2			
38·0–38·3	—	—	71·7	
38·3–39·0	—	47·9		
40·0	19·7			
42·0	—	—	54·1	76·8
42·0–42·2	—	29·7		
42·3–42·2	11·4			
45·0	—	—	24·0	49·4
45·2–45·4	—	14·2		
47·6–47·2	—	—	—	24·0

On plotting as ordinates the percentage of maltase hydrolysed, and as abscissæ the corresponding mean temperature, these numbers give the graphical representation of fig. 2.

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* 'Bull. Soc. Chim.,' (3), vol. 35, p. 1285 (1906).

This figure shows that for the acidity in question of the medium addition of more enzyme has merely increased the rate of hydrolysis without changing in any way the optimum temperature, the latter remaining fixed in the neighbourhood of 35·5°, and that no matter what the enzyme concentration. This result was quite unsuspected.

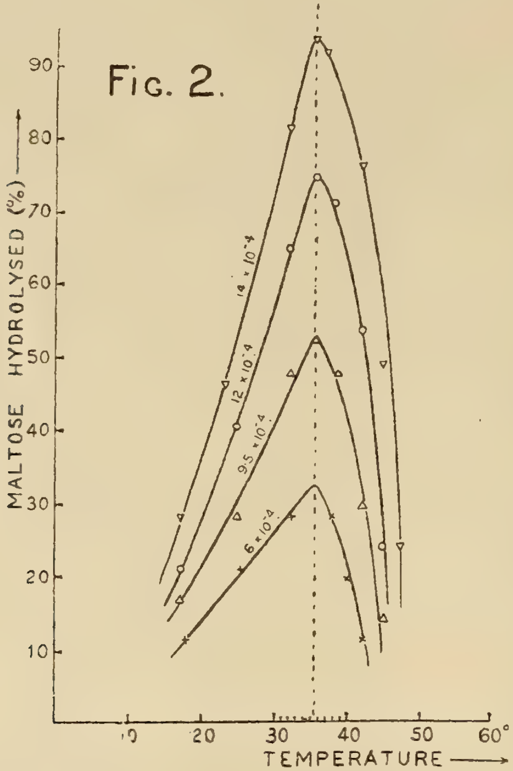

Fig. 2.—Subtrate concentration M/20. Enzyme concentrations 6×10^{-4} to 14×10^{-4} grm. per cm. Reaction of medium—acid.

As, however, it recalls the corresponding course of events for the natural reaction—the enzyme preparation being dissolved in pure water, without the addition of acid as above—fig. 3 is here reproduced for convenience of reference from former work,* carried out in this respect with the same preparation of *maltase.* In the experiment, of which fig. 3 is the graphical *résumé,* the enzyme was employed over a wider range of dilutions than in the foregoing acid experiment: in concentrations varying between 2×10^{-4} grm. and 20×10^{-4} grm. per cm.^{3}, while the subtrate concentration and the duration of the experiment were the same. Under these circumstances, an optimum temperature of +47° is seen to obtain, and that no matter what the enzyme concentration.

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* Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 88, pp. 258–263 (1914), and *loc. cit.*

Figs. 2 and 3, in the constancy of the optimum temperatures which they respectively record, are very striking and indicate clearly the *rôle* of the chemical reaction, or H^+ concentration, of the medium in fixing the optimum temperature of the ferment.

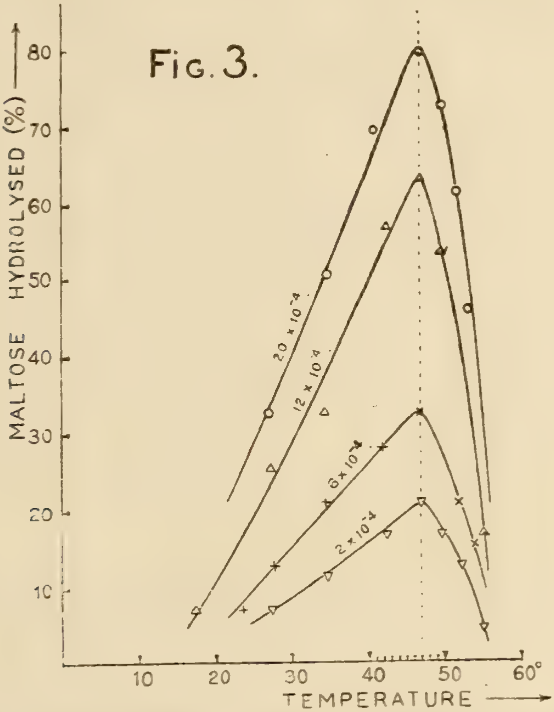

FIG. 3.—Subtrate concentration M/20. Enzyme concentrations 2×10^{-4} to 20×10^{-4} grm. per cm.^{3} Reaction of medium—natural reaction.

Actual measurements were made of the H^+ concentration, by Sorensen's calorimetric method,* for the different enzyme dilutions employed in the two foregoing series of experiments. For the natural reaction series (fig. 3), the H^+ concentrations of the different dilutions were found to be all identical, while the same was found to be true of the acid series (fig. 2), the two distinctive values of P_H thus obtained being respectively as indicated in fig. 1.

The variation, then, of the optimum temperature of an enzyme with the chemical reaction, or H^+ concentration, of the medium, previously established by us,* and its actual fixation for any given H^+ concentration, as shown to-day, constitute, as will readily be understood, but different expressions of the same phenomenon, being a consequence the one of the other.

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* 'C. R. Lab. de Carlsberg,' vol. 8, p. 148 (1909).

The bearing of these results on the general law of the independence of the optimum temperature of an enzyme of the concentration of the latter, will not have escaped notice. We see in them but instances of the working of this law, which they throw a new light upon, in indicating that in its original statement it is stated too briefly,† and now requires amplification. In consequence, re-stated, it becomes: the optimum temperature of any ferment, or ferment function, occurring in a given enzymic preparation is independent of the concentration of the enzyme, the duration of the action and *the chemical reaction—or* H^+ *concentration—of the medium* being constant.

The question asked at the beginning of the investigation thus finds itself answered; and, if in a manner different from the limited sense in which it was formulated, still the answer is none the less instructive, in the light which it throws on a general aspect of the mechanism of enzyme action.

The Effect of Certain Dietary Deficiencies on the Suprarenal Glands.

By C. H. Kellaway, Foulerton Student of the Royal Society.

(Communicated by W. B. Hardy, Sec.R.S. Received August 13, 1920.)

[Plate 1.]

During recent years McCarrison (1919 *a*, *b*, and 1920) has published a series of important papers dealing with the effect of deficient diets on the various organs of the body. One very striking result which he has described was a great enlargement of the suprarenal glands, with pronounced increase in their content of adrenaline, in pigeons fed on polished rice and suffering from the consequent polyneuritis. McCarrison's experience led him to put forward the tentative suggestion, that the increased content of adrenaline might be significant of increased output of adrenaline during the development of the disease, and that this might account for the occurrence of œdema both in experimental polyneuritis produced in pigeons and in the wet form of human beri-beri. In certain later experiments on pigeons fed on polished rice with the addition of butter and onions, he found that the polyneuritis, which was quickly produced on this diet, was only rarely accompanied by œdema.

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* Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 88, pp. 408–417 (1915).

† Arthur Compton, 'Roy. Soc. Proc.,' B, 87, pp. 245–254 (1914); 'Ann. Inst. Past.,' vol. 28, pp. 866–878 (1914).

There were several points in McCarrison's suggestive observations which seemed worthy of further investigation. In the first place it was not clear whether the effect of an exclusive diet of polished rice on the adrenals of pigeons was due to specific deficiency of the accessary factors concerned with the development of polyneuritis, or to the more general deficiency of protein, fat or salts entailed by such a diet. In the second place, assuming the effect to be a specific one, and McCarrison right in suggesting a causal connection between excess of adrenaline and the appearance of œdema, corresponding to the wet form of beri-beri, it seemed that the investigation of this connection might lead to results of more general pathological importance. While the work was in progress McCarrison himself found reason to withdraw the suggestion, which he had put forward at an earlier stage, so that the position to which my own experiments have led me is in substantial agreement with that which McCarrison now holds. At the same time it seems worth while to put on record the experiments made on this aspect of the question.

Finally, McCarrison's observations on the occurrence of similar adrenal changes in inanition, the apparent relation between their incidence and the fall of body weight in pigeons fed on polished rice, and his opinion based on the study of histological material, that the enlargement of the cortex is disproportionately large compared with that of the medulla in the adrenals of these birds, suggested that a partial explanation of the enlargement might be found in the storage in the cortex of the gland of lipoids set free by the breaking down of body tissues. This hypothesis has been tested by observations on the cholesterol content of the blood and adrenals of normal and experimental pigeons. The further possibility that enlargement produced by deposition of lipoids in the cortex might be associated with increased residual content of adrenaline has been tested by feeding rabbits and pigeons on diets containing excess of cholesterol.

Methods.

The pigeons used in these experiments were Blue Rocks, less than one year in age. In order to produce polyneuritis I have used artificial feeding with polished rice, except in one experiment, which will be referred to later. In the birds which were artificially fed, polished rice to an amount of 15–20 grm. was introduced daily into the crop. Great care was taken to prevent the birds so fed from becoming "crop-bound." When too much rice was found in the crop at the time of feeding, one or two feeds were omitted till the crop was empty.

When other substances were added to the dietary, they were introduced into the crop by means of a graduated syringe with a catheter tube attached. "Marmite" was given in two strengths, 1 grm. in 2 c.c. or 2 grm. in 3 c.c., made up with tap water. Olive oil and cod liver oil were administered in the same way. Fat-free casein was given in the form of pellets, rolled from a thick paste of the protein with water, and dried.

In the first two weeks of feeding the birds retained the rice which was placed in their crops, but later some of them tended to reject a portion, though not all, of the feed. This tendency was only noticed in pigeons fed on polished rice without the addition of "marmite." When "marmite" was given, the rice was never rejected.

The initial and final weights and the cloacal temperature were recorded. The weighings were made with the crop empty. The final weighings were made after death, and when necessary, the crop was opened and emptied.

The birds were killed by a uniform method, the neck being rapidly dislocated. In the earlier experiments a culture was taken from the heart's blood in every case, but as these cultures were invariably sterile, this precaution was discarded in the later experiments. After death the adrenals were quickly dissected out, carefully cleaned from surrounding tissue and dried with filter paper before weighing. A saline extract of the glands was made, which was acidified, boiled, filtered, and made up to a known volume of 3 c.c. to 5 c.c. This solution was always perfectly clear and suitable either for colorimetric or physiological estimation of adrenaline.

For the estimation of adrenaline the routine method used was that of Folin, Cannon and Dennis (1912). It was found convenient in making up the standard uric acid solution to use twice as much 4 per cent. lithium carbonate solution as that recommended by these authors (vide Seidell, 1913).

Folin, Cannon and Dennis compared the results obtained by their method with those given by the physiological estimation devised by Elliott (1912) in a good many different animals, but it was thought advisable, in view of the important part played by uric acid in avian metabolism, to institute a further comparison on the suprarenal glands of normal and experimental birds. The results of such comparison showed clearly that for purposes of this investigation the figures obtained by the colorimetric method indicated the actual content of adrenaline, and that no appreciable error was introduced by the presence of other substances which might be capable of giving the colorimetric reaction. Table I gives some of the results, and I have introduced a typical tracing of an estimation on a pithed spinal cat (fig. 1).

Table I.—Comparison of Methods of Estimating Adrenaline.

Initial weight in grammes.	Weight of adrenals in milli-grammes.	Weight of adrenaline in milligrammes determined physiologically.	Weight of adrenaline in milligrammes determined colorimetrically.	Remarks.
350	21·0	0·028	0·030	Normal.
450	19·0	0·028	0·031	”
320	31·0	0·095	0·095	” (some time in the laboratory).
319	16·0	0·045	0·046	”
319	27·0	0·060	0·056	”
440	67·0	0·105	0·103	Pigeon with polyneuritis fed on polished rice.
430	71·0	0·086	0·090	” ”
420	55·0	0·168	0·159	” ”
345	20·0	0·105	0·115	Pigeons without symptoms fed on polished rice + 1 grm. caseine + 1 grm. marmite daily.
340	25·0	0·133 (fig. 1).	0·128	

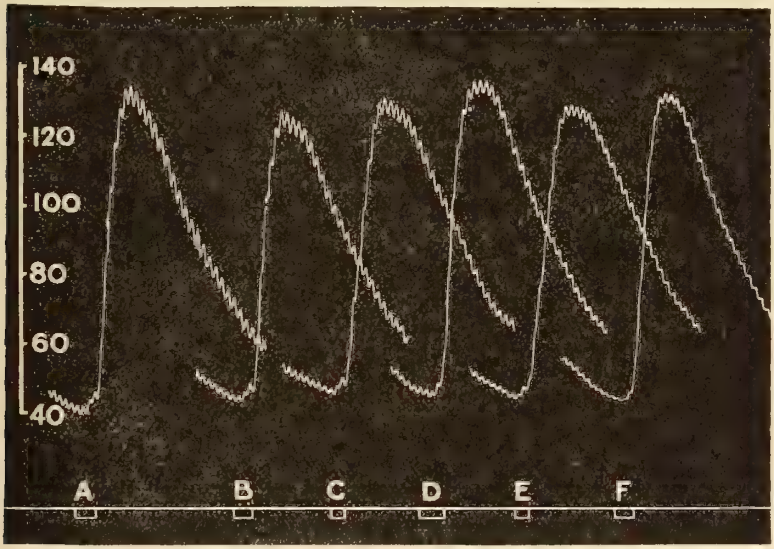

Fig. 1.—Physiological estimation of adrenaline in the suprarenal glands of a pigeon fed on polished rice + 1 grm. marmite and 1 grm. of casein daily. C and E = response to 0·35 c.c. of extract (total volume = 5·1 c.c.). A, D, and F = response to 0·0098 mgrm. B, to 0·0084 mgrm. of adrenaline. Total content of glands = 0·133 mgrm. of base.

For quantities of the order estimated the differences observed are within the limits of experimental error.

In a certain number of birds the adrenals were estimated separately, and in normal birds, even when there was some difference in weight between the two adrenals, the content of adrenaline appears to be identical in the two organs (*cf.* Elliott (1912)). In experimental polyneuritis, however, there is often a large difference in weight between the two adrenals, and there appears to be some corresponding difference in content of adrenaline.

Observations on Normal Pigeons.

In the early stages of this work I was not aware of the variations which occur in the adrenal-content of pigeons in captivity fed on a normal diet, and in consequence some of my first observations on the effect of diet on the suprarenals were, in this respect, imperfectly controlled.

The data which are presented here do not enable me to state definitely how far lack of exercise and prolonged caging, and how far seasonal variation account for these differences in the amount of adrenaline in the glands of normal pigeons. In Table II the average results obtained from three series of normal birds, kept caged for varying periods, are given. The extreme variations of individual birds are indicated by the maximal and minimal figures placed beneath each average. Birds whose organs showed any gross pathological changes at autopsy were discarded.

Table II.—Pigeons on Normal Diet.

	Number of series.	Period of time during which birds were caged.	Number of birds in series.	Body weight in grammes.	Weight of adrenals in milli-grammes.	Weight of adrenals per kilo-gramme of body weight in milli-grammes.	Weight of adrenaline in milli-grammes.	Weight of adrenaline per kilo-gramme of body weight in milli-grammes.
Average	(1)	March 1	13	*318·4*	*25·2*	*79·1*	*0·051*	*0·160*
Maximal		to		352	34·5	113·5	0·096	0·300
Minimal		March 15		289	16	50·2	0·033	0·112
Average		April 5	4	*304*	*18·5*	*60·9*	*0·077*	*0·225*
Maximal		to		306	22	71·9	0·101	0·330
Minimal		May 28		301	15	49·3	0·035	0·115
Average	(11)	May 5	8	*284*	*24*	*84·5*	*0·096*	*0·338*
Maximal		to		314	32	101·9	0·129	0·411
Minimal		June 30		248	15	60·5	0·064	0·258

The striking difference between the average figures for content of adrenaline per kilogramme of initial body weight in Series 1 and 11, *i.e.*, 0·160 and 0·338 mgrm., makes it clear that in estimating the effect of any diet on the suprarenals the influence of caging and seasonal change must be excluded by comparison with birds on normal diet kept for the same period under identical conditions.

Observations on Pigeons fed with Polished Rice.

As an initial experiment two series, each of twelve birds, were fed, those of one series artificially with polished rice, while those of the other were allowed to feed themselves on polished rice which had been autoclaved for 1½ hours at 130° C. The artificially fed birds of Series 2, with two exceptions, developed polyneuritis in from seventeen to twenty-nine days. In Series 3 six birds showed well-marked symptoms between the sixteenth and twenty-second days; one was found dead on the ninteenth day with multiple liver abscesses and was excluded from the record, and the remaining five were killed on the twenty-fourth and twenty-fifth days without having shown symptoms of polyneuritis. In this experiment the birds were killed and the estimations made as soon as the diagnosis of polyneuritis was certain.

The general *post mortem* findings resembled those described in detail by McCarrison (1919 *a*), except with regard to the incidence of "œdema." Only three pigeons showed any sign of this. In pigeon 1, which was fed artificially for twenty-three days, the pectoral muscles were definitely more moist than normal, and the peritoneum and pericardium contained very small quantities of fluid. Pigeon 7, which was fed in the same way for twenty-three days, showed the same picture at autopsy, and pigeon H, which fed naturally on polished rice for twenty days, had a little fluid in the pericardium at death.

The average results for Series 2 and 3 are given in Table III. In Series 3 the birds have been divided into two groups and the results averaged separately, group (*a*) consisting of birds without symptoms of polyneuritis, and group (*b*) of those which acquired symptoms in from sixteen to twenty-two days.

The birds of this series which, after the first few days, ate very little, lost

Table III.—Pigeons Fed on Polished Rice.

	Number of series.	Number of birds in series.	Number of days on diet.	Initial weight in grm.	Final weight in grm.	Weight of adrenals in mgrm.	Weight of adrenals per kilog. initial weight in mgrm.	Weight of adrenaline in mgrm.	Weight of adrenaline per kilog. of initial weight in mgrm.
Average ...	(2)	11	*22·8*	*371·8*	*293·0*	*63·9*	*172·0*	*0·132*	*0·356*
Maximal ...			31	460	361	86	277·4	0·314	0·683
Minimal ...			17	280	200	44·5	117·1	0·058	0·193
Average (a)	(3)	5	*24*	*364*	*229·8*	*38·0*	*104·4*	*0·082*	*0·225*
Average (b)		6	*18·7*	*350*	*256·7*	*39·3*	*112·3*	*0·102*	*0·291*
Average total		11	*21*	*356*	*244*	*38·7*	*108·7*	*0·093*	*0·261*

weight more rapidly than the artificially fed birds of Series 2. As under these conditions they were largely living on their own tissues, they may have derived thence an amount of accessory substance sufficient to delay the appearance of polyneuritis.

In Series 2 the average weight of the adrenals was 172·0 mgrm., and of the adrenaline 0·356 mgrm. per kilogramme of the original body weight. In Series 3 these figures were 108·7 mgrm. and 0·261 mgrm. The corresponding figures for the first group of normal pigeons given in Table II were 79·1 mgrm. and 0·160 mgrm. The results from these normal pigeons cannot, however, be regarded as strictly controlling this experiment so far as the content of adrenaline in the suprarenals is concerned, since the birds which gave them had not been caged for the same length of time as those fed on rice.

This experiment, and all subsequent ones, confirmed McCarrison's findings in regard to adrenal hypertrophy in avian polyneuritis. The figures obtained for the store of adrenaline in this experiment, though nearly double those yielded by normal birds investigated at the same time, were not nearly so high as those obtained later in other normal birds. This, in itself, seemed to indicate that changes in the adrenaline-content of the suprarenals are not necessarily of importance in the development of avian polyneuritis. Further evidence in this direction was supplied by later observations.

A further series of six pigeons which were fed with polished rice, and which were killed for analysis at the same time as Series 11, in Table II showed a large increase in the store of adrenaline when compared with the normal birds, which in this case provided an accurate control.

The results of this series are given in full in Table IV.

Table IV.—Series 12. Pigeons Fed on Polished Rice.

Distinguishing number.	Number of days on diet.	Initial weight in grm.	Final weight in grm.	Weight of adrenals in mgrm.	Weight of adrenals per kilog. of original weight in mgrm.	Weight of adrenaline in mgrm.	Weight of adrenaline per kilog. of original weight in mgrm.
0	32	270	238	45	166·7	0·168	0·622
1	32	310	257	35	112·9	0·126	0·406
2	19	310	247	48	154·8	0·240	0·774
3	32	320	259	41	128·1	0·199	0·622
4	28	300	270	52	173·3	0·167	0·557
5	31	310	265	66	212·9	0·161	0·519
Average ...	—	303	256	48	158·4	0·177	0·584

This experiment was rigidly controlled not only by observations on normal pigeons kept under identical conditions, but also upon birds fed on polished rice with the addition of a daily ration of "marmite," and showed conclusively that there is a definite increase of adrenaline in the suprarenals of birds fed on polished rice with consequent polyneuritis. The significance of this increase will be discussed later.

The Addition of other Substances to the Diet of Polished Rice.

According to Kellner's analyses, quoted by König (1893), polished rice contains 12·55 per cent. of water, 7·88 per cent. nitrogenous substances, 0·53 per cent. of fat, 77·8 per cent. of non-nitrogenous extractives and 0·78 per cent. of ash. A diet of polished rice is therefore deficient in protein, fat and salts as well as in the accessory substances—fat soluble A and water soluble B. The mixed pigeon seed on which my normal birds were fed contained 2·33 per cent. of nitrogen (as against 1·24 per cent. for polished rice), and 2·2 per cent. of ether soluble constituents.

A series of experiments were now made to show the effect on the hypertrophy of the adrenals and their content of adrenaline, of adding some of the deficient constituents to the diet of polished rice.

The first group of experiments lasted from April 6 to May 7. In them the effect of the addition of "marmite" (a commercial extract of yeast), and of a fat deficient in all accessory factors was tried. Chick and Hume (1917) had previously shown that the daily dose of commercial yeast extract required to protect pigeons of 300–400 grm. weight from polyneuritis when fed on polished rice was from 1·0 to 2 grm. Four series of birds were started on April 6—Series 4, 5, 6, and 7. The first were given 1 grm. of "marmite" daily, the second 0·75 grm. of "marmite," the third 1 grm. of "marmite" and 1 c.c. of olive oil and the fourth 1 c.c. of olive oil but no "marmite," in addition to their daily ration of polished rice. The average results of the adrenal estimations in these series are given in Tables V and VI.

A further series of experiments were made between April 29 and the end of May. Three series of birds were fed with the addition, in Series 8 of 1 grm. of fat free casein, in Series 9 of 1 grm. of fat free casein and 1 grm. of "marmite," and in Series 10 of 1 c.c. of cod liver oil daily. The results of these experiments are given in Tables VI and VII. Finally, from the end of May till the end of June, several series of birds were fed, of which we are here concerned with Series 13 (Table V), in which a daily ration of 2 grm. of "marmite" was added to the diet of polished rice.

The Effect of the Addition of "Marmite" to a Diet of Polished Rice.

In order to ascertain the effect produced on the adrenals by the addition of "marmite" to a diet of polished rice comparisons may be instituted between the results of Series 4 and 5 and 6 and 7 recorded in Tables V and VI; between those of Series 8 and 9 in Table VII and finally between the figures given for Series 11, 12, and 13 in Tables III, IV, and V. The final comparison gives clear and definite information and will be discussed first.

In Table V, Series 13, the effect of the daily addition of 2 grm. of "marmite" to the diet of polished rice is shown. This dose is twice the daily amount required to protect the birds from polyneuritis.

The average weight of the adrenals per kilogramme of original body weight is 94·7 mgrm., and that of the adrenaline present in the suprarenals is 0·361 mgrm. as compared with 84·5 mgrm. and 0·338 mgrm. in normal birds (Table II, Series 11) and 158·4 mgrm. and 0·584 mgrm. (Table IV, Series 12) in birds with polyneuritis, observed at the same time and kept under the same conditions. This amount of "marmite" in addition to affording complete protection against the disease, enables the birds to gain in weight, and prevents the occurrence of adrenal hypertrophy and also of any definite increase above the normal in the store of adrenaline.

In Series 4 with a daily ration of 1 grm. of "marmite" (Table V) and in Series 5 with a daily dose of 0·75 grm. this result is also indicated, though there are no figures for normal birds which are strictly comparable with those obtained from these series.

The effect of yeast extract, in preventing the adrenal changes caused by a diet of polished rice alone is clearly shown; in the succeeding sections it will appear that it is much less effective in this direction when the basic diet contains protein or fat, free from accessory factors, as well as polished rice.

The Effect of the Addition of a Fat free from Accessory Factors to the Diet of Polished Rice.

As a fat free from accessory factors olive oil was selected and Series 6 and 7 (Table VI) show the effect of a daily ration of 1 c.c. of olive oil together with 1 grm. of "marmite," and 1 c.c. of olive oil without any "marmite," to the diet of polished rice. The pigeons fed on olive oil and rice without "marmite" (Series 7) exhibited symptoms of polyneuritis in between fifteen and thirty days.

One pigeon of this series, which had a higher-adrenal content than the others, had a trace of fluid in the pericardium at autopsy, and, in addition, the pectoral muscles were moister than normal.

Table V.—Effect of the Addition of "Marmite" to the Diet of Polished Rice.

	Number of series.	Number of birds in series.	Daily ration of marmite.	Number of days on diet.	Initial weight in grammes.	Final weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenals per kilogramme of initial weight in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of initial weight in milligrammes.
Average	(4)	6	1 grm.	30 (April 6 to May 7)	*312*	*349*	*20·3*	*65·1*	*0·087*	*0·279*
Maximal							26	84·7	0·105	0·356
Minimal							15	50·0	0·070	0·233
Average	(5)	6	0·75 grm.	30 (April 6 to May 7)	*306*	*322*	*26·0*	*85·0*	*0·109*	*0·356*
Maximal							31	101·1	0·129	0·469
Minimal							23	67·3	0·077	0·245
Average	(13)	6	2 grm.	30 (May 31 to June 30). In laboratory from May 5	*302*	*358*	*28·6*	*94·7*	*0·109*	*0·361*
Maximal							40	125·0	0·150	0·490
Minimal							23	71·9	0·084	0·269

Table VI.—The Effect of the Addition of Fat to the Diet of Polished Rice.

	Number of series.	Number of birds.	Diet.	Period for which diet given.	Initial weight in grammes.	Final weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenals per kilogramme of initial weight in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of initial weight in milligrammes.
Average	(6)	6	Polished rice + 1 grm. "marmite" + 1 c.c. olive oil	April 6 to May 5	*325·7*	*339·8*	*26·8*	*82·3*	*0·132*	*0·405*
Maximal							29	95·9	0·167	0·572
Minimal							25	75·5	0·092	0·309
Average	(7)	6	Polished rice + 1 c.c. olive oil	April 6 to May 5	*301*	*263*	*55·2*	*183·4*	*0·136*	*0·452*
Maximal							77	247·6	0·156	0·529
Minimal							43	145·8	0·092	0·296
Average	(10)	5	Polished rice + 1 c.c. cod liver oil	April 29 to May 20	*306*	*263*	*31·6*	*103·3*	*0·134*	*0·438*
Maximal							53	182·7	0·165	0·569
Minimal							22	70·9	0·108	0·354

It is evident that this dose of "marmite," when added to a diet of polished rice and oil, though it prevents the onset of symptoms and the enlargement of the suprarenals, is not enough to prevent some increase in the amount of adrenaline. The deficiency of fat in a dietary of polished rice has obviously no causal relation either to the hypertrophy of the adrenals or to increase in the content of adrenaline produced by feeding with such a diet.

The Effect of the Addition of a Fat Rich in Fat Soluble A, to the Diet of Polished Rice.

Although there is no evidence that fat soluble, A, is a necessary constituent of the normal diet of pigeons, the effect of the addition to the diet of polished rice of cod liver oil, a fat rich in this accessory factor, was investigated. Series 10 (Table VI) gives the results obtained.

Four out of the five birds in this series were killed on the twentieth day without having exhibited any symptoms of polyneuritis, though they showed some fall of body weight; there was no lowering of their cloacal temperature, and they were indistinguishable from normal birds. One was kept on the diet till the thirty-fifth day, and was then at the point of death, with extremely low cloacal temperature, great weakness and wasting, but with no definite symptoms of polyneuritis. This bird had been emptying its crop shortly after feeding, a tendency which was marked in all the birds of this group.

These pigeons had been caged for a month before the experiment was started, and the figures for weight of adrenals and of adrenaline per kilogramme of original body weight, *i.e.*, 103·3 mgrm. and 0·438 mgrm., may be compared with those of normal birds kept the same time under identical conditions, *i.e.*, 60·9 mgrm. and 0·255 mgrm. (Table II).

This experiment is complicated by inanition, which McCarrison (1919, *a*) has shown to cause both the suprarenal effects under investigation, but affords no evidence that fat associated with fat soluble A, behaves at all differently from fat free from accessory substances, so far as these suprarenal changes are concerned. It seems possible that the absence of definite symptoms of polyneuritis from these birds may be due to the presence of a trace of water soluble accessory factor in the daily ration of cod liver oil.

The Effect of the Addition to the Diet of Protein free from Vitamines and Fat.

The protein used for this purpose was commercial casein, which, after a preliminary extraction with cold ether, was repeatedly extracted under a reflux condenser with boiling absolute alcohol. This treatment, in addition to removing all the fat, was efficient in removing the water soluble B, as will be shown by the experiment here described.

Two series of birds were fed, with the addition in Series 8 of 1 grm. of fat-free casein, and in Series 9 of 1 grm. of fat-free casein, and 1 grm. of "marmite" daily to the diet of polished rice. The birds of Series 8 rapidly acquired polyneuritis, while those of Series 9 remained normal. Table VII gives the results obtained.

The birds of these series were kept for about a month in the laboratory before the experiment was started, and the figures obtained are comparable with those obtained from the second series of normal birds in Table II.

The effect of the yeast extract in preventing adrenal hypertrophy in pigeons fed on polished rice is evidently not due to any protein or protein derivatives, which are present in small amount in such a ration, but to the water soluble B, which is present, though the influence of the salts in such a yeast extract is not excluded.

On the other hand, the addition of "marmite" to the casein-rice diet does not perceptibly prevent increase of the store of adrenaline, the figures for Series 8 and 9 being nearly identical, though the dose was adequate to prevent such increase in birds fed on a basic diet of rice alone. It becomes increasingly evident that the exhibition of symptoms of polyneuritis is not related to the increase in content of adrenaline, which occurs as a result of feeding pigeons on polished rice.

The Histology of the Adrenals of Polyneuritic Pigeons.

McCarrison (1919, *a*) has already made some important observations on the histological appearances presented by the adrenals of pigeons fed on polished rice. The chief points to which he has drawn attention are: the disproportionate enlargement of the cortex as compared with that of the medulla in these glands; the presence of congestion, which is neither sufficiently constant nor pronounced wholly to explain the enlargement; the occasional broken down appearance of the central part of the gland, together with general nuclear changes in both cortex and medulla, which suggest degeneration; and, finally, the degeneration which is sometimes present in sympathetic ganglia adherent to the gland.

I have examined the adrenals of a few normal and experimental pigeons in order to see if any further light could be thrown on the cause of the changes which occur in them as a result of deficiency diets. I have used two special methods of staining, the bichromate method of Kohn as described by Elliott and Tuckett (1906), using Scharlach, R, as a selective counterstain for the

Table VII.—The Effect of the Addition of Casein to the Diet of Polished Rice.

	Number of series.	Number of birds.	Diet.	Period for which diet given.	Initial weight in grammes.	Final weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenals per kilogramme of initial weight in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of initial weight in milligrammes.
Average	(8)	6	Polished rice + 1 grm. casein	April 29 to May 20	*328*	*285*	*35·5*	*108·2*	*0·122*	*0·372*
Maximal							47	134·2	0·153	0·483
Minimal							23	71·9	0·091	0·283
Average	(9)	6	Polished rice + 1 grm. casein + 1 grm. marmite	April 29 to May 20	*313·3*	*351·7*	*24·3*	*77·6*	*0·115*	*0·367*
Maximal							35	120·7	0·143	0·493
Minimal							15	55·5	0·077	0·285

Table VIII.—Estimation of Cholesterol by the Colorimetric Method.

Amount of cholesterol taken	2·52	2·64	2·76	2·88	3·12	3·24	3·36	3·48
Amount of cholesterol estimated	2·50	2·61	2·70	2·73	3·12	3·24	3·24	3·42

fats and lipoids of the cortex, and the osmic vapour method more recently used by Cramer (1918). Some of the frozen sections, prepared after treatment with bichromate solution, were stained with hæmatoxylin and eosin.

In addition to the changes described by McCarrison, most of which I have also noticed, there are two additional points which may be of importance. In the first place, the cortical enlargement appears to be accompanied, not only by degenerative nuclear changes, but also by a loss of definition of the lipoid granules present in it. This is well seen in frozen sections stained with Scharlach, R.

Secondly, the enlargement of the cortex appears to interrupt the continuity of the normal network of medullary substance, and gives it an appearance on section of a number of isolated nodes surrounded on all sides by swollen cortical tissue, and only connected with each other by fine strands of tissue. This appearance is well shown in the osmic vapour preparations which have been kindly photographed for me by Mr. Barnard (Plate 1, figs. 2 and 3).

Finally, it is of interest to record that the adrenals of pigeons fed on polished rice, with the addition of a daily ration of "marmite," differ histologically in no important respect from those of normal birds.

These histological appearances suggest two possible ways in which the output of adrenaline into the blood stream might be hampered or prevented in pigeons on such deficiency diet. The presence of degenerated sympathetic ganglia described by McCarrison indicates the possibility that the splanchnic fibres to the adrenals might also be degenerated, though I have not been able to show that this is the case. If this were so, the output of adrenaline from the glands would be greatly diminished (Elliott, 1912). On the other hand, the cortical swelling might conceivably act mechanically, by hampering the venous outflow from the gland, and so diminishing the output of adrenaline.

The Significance of Adrenal Enlargement of Pigeons fed on Polished Rice.

Various factors may come into play in causing the adrenal enlargement which occurs in pigeons fed on polished rice. Histological observations seem to exclude congestion of the glands as a constant factor in the production of this effect. Apart from congestion, some œdema of the gland tissues is consistent with the histological appearances presented by them, and part of the increase in weight is explained by an increase in the water-content of the adrenals.

The weight of the freshly dissected-out adrenals of a normal pigeon was 23·0 mgrm., and when these were dried to constant weight over sulphuric acid *in vacuo* they weighed 7·0 mgrm. The percentage of water in these

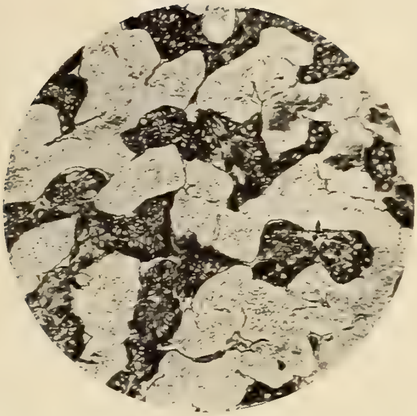

FIG. 2.—Normal adrenal of pigeon. Osmic vapour preparation (×140 diameters) showing the continuity of the medullary network.

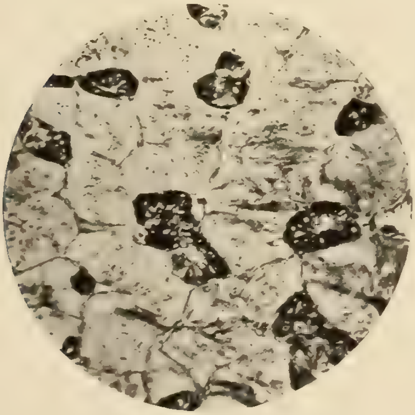

FIG. 3.—Adrenal from polyneuritic pigeon (×140 diameters) showing excessive cortical enlargement and interruption of medullary network.

glands was 69·6 per cent. The adrenals of a polyneuritic pigeon similarly treated gave wet and dry weights of 41·0 mgrm. and 10·0 mgrm., and the percentage of water in them was 75·6 per cent.

Apart from increase in water-content, the fact that enlargement of the cortex predominated over that of the medulla in the adrenals of pigeons fed on polished rice suggests a further possible reason for the increase in weight of the glands. McCarrison's observations on the occurrence of adrenal enlargement in pigeons suffering from inanition have already been referred to. In his feeding experiment on polished rice, the increase in weight of the adrenals is associated with loss of body weight, and in my own experiments loss of body weight and adrenal enlargement have been almost constantly associated.

Ellis and Gardiner (1912) have shown that in rabbits, which live normally on a diet poor in cholesterol, inanition causes a rise in the content of cholesterol in the blood, and Anitschow and Chalatow (1913) produced adrenal enlargement in these animals by feeding with excess of cholesterol. It was conceivable, therefore, that the enlargement of the suprarenals, which occurs in the production of experimental polyneuritis, was the result of inanition and was due to the storage in the adrenal cortex of lipoids which had been set free by the breaking down of the body tissues. The validity of this hypothesis was tested by observations on the cholesterol-content of the adrenals of normal and polyneuritic pigeons. For this purpose the colorimetric method of Grigaut (1911) was used and some concurrent estimations of the cholesterol-content of the blood of such pigeons were also made by the same method. Such colometric determinations were found to be most accurate when the standard contained approximately the same amount of cholesterol as the solution to be tested. The observations in Table VIII show the degree of accuracy of colorimetric estimations when this precaution was taken.

The cholesterol extracted from the adrenals was compared with a standard containing 1·2 mgrm. of the pure substance, while for blood a standard containing 3 mgrm. was used. The chief difficulty in making the estimations was caused by the presence in the blood and gland extracts of organic matter which altered the tint, making it greener than the standard. Screens of amber glass were found to be of use in making the colour comparison in such cases.

The average results obtained in normal and polyneuritic pigeons are given in Table IX.

In pigeons fed on polished rice with extreme symptoms of polyneuritis there is an increase in the percentage of cholesterol in the blood, but no

Table IX.—The Cholesterol-content of the Blood and Adrenals of Normal and Polyneuritic Pigeons.

	Diet.	Number of days on diet.	Initial body weight in grm.	Weight of adrenals in mgrm.	Cholesterol-content of adrenals in mgrm.	Percentage of cholesterol in adrenals.	Weight of cholesterol on 1 c.c. of blood in mgrm.
Average	Normal	—	*314·6*	*25·5*	*0·70*	*2·9*	*1 66*
Maximal				32·0	0·88	3·8	2·46
Minimal				21·0	0·44	2·0	1·06
Average	Polished rice	*18*	*320*	*47·7*	*0·79*	*1·9*	*3·06*
Maximal				66·0	1·34	3·3	3·69
Minimal				32·0	0·35	0·67	2·40

evidence of storage in the suprarenals. These results do not lend any support to the hypothesis of lipoid storage as far as cholesterol is concerned. Some observations were also made on rabbits and pigeons fed on a normal diet with the addition of cholesterol, in order to ascertain if the hypercholesteræmia produced in this way was associated with increase in the content of adrenaline in the glands.

Feeding Experiments with Cholesterol.

Eleven healthy rabbits, two months old, were selected for experiment. Of these, six were fed daily through a catheter with 0·1 grm. of pure cholesterol dissolved in 2·5 c. c. of olive oil. All the rabbits had the same basal dietary, consisting of bran, oats, and occasional greens. The experiment was continued for nearly seven weeks. All the rabbits remained healthy and gained weight, except one of the experimental animals, which became paraplegic after forty-two days' feeding, and which was discarded. At autopsy the animals fed with excess of cholesterol showed aortic lesions like those described by Anitschow and Chalatow, but only two had any adrenal hypertrophy.

Before the rabbits were killed, a sample of blood was taken from the ear for cholesterol estimation. The adrenals were carefully dissected out *post mortem*, weighed, and the residual adrenaline was estimated colorimetrically.

The average results obtained from the two series of rabbits are given in Table X.

There is a small increase in the content of adrenaline of the rabbits in which hypercholesteræmia had been produced and maintained, above the average content of normal rabbits investigated at the same time. A more rigid control would have been provided had these latter animals been fed daily with 2·5 c.c. of olive oil without cholesterol.

Table X.—The Effect of Excess of Cholesterol in the Diet of Rabbits.

	Diet.	Number of rabbits in series.	Number of days on diet.	Initial body weight in grammes.	Final body weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of final weight in milligrammes.	Weight of cholesterol per cubic-centimetre of blood in milligrammes.
Average	Normal	5	—	—	*1040*	*113·2*	*0·219*	*0·218*	*0·91*
Maximal						170	0·280	0·333	1·21
Minimal						86	0·175	0·159	0·63
Average	Normal +0·1 grm. cholesterol in 2·5 c.c. olive oil	5	*41*	*780*	*1160*	*218*	*0·305*	*0·258*	*3·50*
Maximal			46	960	1400	328	0·469	0·347	5·34
Minimal			33	605	900	128	0·201	0·163	0·90*

* No cholesterol given for forty-eight hours before death, at which time the estimation was made.

Table XI.—The Effect of Addition of Cholesterol to the Normal Diet of Pigeons.

	Period in laboratory.	Period of feeding with excess of cholesterol.	Initial weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenals per kilogramme of initial weight in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of initial weight in milligrammes.
Average	April 5 to May 31	April 29 to May 31	*325*	*16·0*	*49·2*	*0·105*	*0·323*
Maximal			360	18	57·1	0·142	0·406
Minimal			280	14	38·5	0·084	0·252

Six pigeons, which were fed on a normal diet with the daily addition of 1/25 grm. of cholesterol dissolved in 1 c.c. of olive oil, and which were kept for the same period and under the same conditions as the second series in Table II, were also investigated. The results are given in Table XI.

Here again there is an increase in the content of adrenaline, though the control would be more accurate if the normal birds had had olive oil in addition to their normal diet.

The production of hypercholesteræmia in normal rabbits and pigeons appears to be associated with a small increase in the content of adrenaline in the suprarenals.

The Significance of Increased Content of Adrenaline in the Suprarenals of Pigeons fed on Polished Rice.

The adrenaline in the suprarenal glands represents the balance between production and secretion into the blood stream. It is of course possible that the increased katabolism induced by feeding pigeons on deficient diets may provide more of the precursor or precursors from which adrenaline is built up by the gland, but this must remain a mere speculation. On the other hand, the evidence provided both by the present experiments and by the more extensive work of McCarrison suggests strongly that the increase of adrenaline in pigeons on deficiency diet is chiefly due to diminished output. The large increase which occurs in pigeons as a result of restriction of activity by caging, the increase which results from inanition and the association of such increase with the diminution of muscular and general metabolic activity in pigeons fed on deficient diets, whether polyneuritis were produced thereby or no, all point in the same direction.

The picture presented by pigeons on a deficient diet is in accord with this view. The progressively falling body temperature, the ruffling of the feathers, which do not lie down closely as would be expected if large quantities of adrenaline were being turned out into the blood stream, and the sluggishness exhibited by such birds, are all consistent with diminished output. The hyperglycæmia observed in pigeons fed on polished rice, which as Funk (1920) has suggested, might be accounted for by increased output of adrenaline, may easily be explained by the diminished use of sugar by the tissues together with the large excess of carbohydrate in the diet.

Whether the diminished output is itself due in the main to depression of metabolism, or whether the nervous and mechanical factors already discussed are chiefly concerned, is a matter on which no evidence is available.

In the present experiments the occurrence of increase in the store of adrenaline in normal birds, which in some cases exceeded the amounts found in polyneuritic birds examined at a different time, makes it seem likely that, whatever the exact significance of the increase may be, it is not causally related to the polyneuritis which occurs in dietaries deficient in water soluble B. Further evidence in this direction is furnished by the experiments on feeding with casein and "marmite," and casein alone, in addition to polished rice, which yielded almost identical results for the store of adrenaline in two series of birds examined at the same time.

The Incidence of Œdema in Experimental Polyneuritis.

In my series of experimental pigeons there were very few examples of the œdema described by McCarrison. Of fifty birds with polyneuritis, only four presented signs of this œdema at autopsy. The œdema occurred either as a wetness of the pectoral muscles or as an effusion of fluid into the pericardium and peritoneum. There were no instances of œdema in the lungs or of the fatty band of tissue round the auriculo-ventricular junction on the surface of the heart. All but one of these œdematous birds gave values for the content of adrenaline which were higher than those given by birds in the same series. The association of œdema with very high content of adrenaline may possibly be explained by œdema of the glands themselves. It is more likely, however, that the factors which operate in producing œdema in the ill-nourished tissues of these birds are the same as those which give rise to diminished use of adrenaline.

McCarrison's early suggestion that the increased store might be evidence of increased output of adrenaline and that this increase might be an important factor in the production of œdema was put to the test of experiment.

Two pigeons—one on a normal diet and the other fed artificially with polished rice—were given daily an amount of adrenaline several times greater than the normal adrenaline-content of the pigeon's suprarenals, *i.e.*, 0·25 mgrm. of adrenaline chloride, which was injected into the pectoral muscles. The experiment lasted from May 31 to June 24, by which time the pigeon on the polished rice dietary was at the point of death with severe symptoms of polyneuritis. Its body weight had fallen from 300 grm. to 265 grm., and its cloacal temperature was 104·5°. It had had severe ataxia for the preceding forty-eight hours and was then unable to walk or fly. At autopsy there were necrotic patches in the atrophic pectoral muscles, but there was no evidence of sepsis and the heart blood was sterile. There was no trace of œdema. The internal organs were all greatly wasted except the adrenals, which weighed 75 mgrm. The left gland, which weighed 37·5 mgrm., contained 0·052 mgrm. of adrenaline and the right gland, which was of equal weight to the left, contained 0·216 mgrm. of cholesterol.

The bird on normal diet was killed on the same day. Its temperature was normal and it was indistinguishable from other normal birds. At autopsy there were necrotic patches in the pectoral muscles but the blood culture was sterile. All the organs appeared to be normal except the pancreas which was somewhat pale. The adrenals weighed 20·5 mgrm. The left gland, which weighed 9·5 mgrm. contained 0·042 mgrm. of adrenaline and the right, which weighed 11 mgrm., contained 0·192 mgrm. of cholesterol.

These birds then, gave adrenaline and cholesterol values for the adrenals, which lay within the limits of values obtained for other normal and polyneuritic pigeons.

In neither of these pigeons was there any œdema and the administration of adrenaline in this way did not appear either to hasten or retard the appearance of symptoms in the bird on the milled rice diet.

It seems unlikely that increased output of adrenaline, even supposing that this could be shown to occur in pigeons fed on polished rice and suffering from the consequent polyneuritis, could contribute to the development of œdema. On the other hand the ill-nourished state of the tissues in such pigeons would predispose them to œdema.

Conclusions.

1. McCarrison's observations on the occurrence of enlargement of the adrenals with increased store of adrenaline in pigeons fed on polished rice, are confirmed.

2. These changes still occur when either protein or fat is added to the diet, but are prevented by the addition of an adequate ration of yeast extract.

3. The addition of such a ration of yeast extract to a basic diet of polished rice with extra fat or protein, does not prevent the increase in the store of adrenaline, though in this case the glands are not enlarged.

4. It is suggested that the enlargement of the adrenals is due partly to congestion and œdema of the gland tissues and partly to the storage in the cortex of the gland, of lipoids set free by the breaking down of body tissues. The investigation of the cholesterol-content of the adrenals of normal and polyneuritic pigeons does not support this theory of lipoid storage, though a well-marked hypercholesteræmia occurs in the latter.

5. The artificial production of hypercholesteræmia in rabbits and pigeons by feeding with cholesterol appears to be associated with a small increase in the adrenaline-content of the suprarenals.

6. The increased content of adrenaline in the suprarenals of birds on deficient diets is attributed to diminished output of adrenaline as a result of lowered body metabolism.

7. The œdema which occurs in some cases of experimental polyneuritis is not due to increased output of adrenaline. Daily administration of adrenaline to birds fed on normal or polished rice does not cause œdema, nor does it accelerate or retard the onset of polyneuritis in pigeons on a polished rice diet.

I desire to express my indebtedness to the Medical Research Council for the facilities given me to carry out this work in their Department of Biochemistry, and especially to Dr. H. H. Dale, F.R.S., for much helpful criticism and advice.

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The Growth of Seedlings in Wind.

By LEONARD HILL, M.B., F.R.S. (Director of the Department of Applied Physiology, National Institute for Medical Research).

(Received October 16, 1920.)

[PLATES 2 AND 3.]

The stunting of plants, and their more twisted, bent, and harder woody nature is seen in wind-swept places, for example, the exposed shores of the Shetland Islands. In Patagonia, where no shrub grows more than a yard or so high, there is sunshine by day and ceaseless wind—an ideal spot for open air treatment. Gardeners use hedges and rush hurdles as wind screens, and secure luxuriant growth in exposed gardens by providing such shelter. The kata-thermometer* gives a measure of the cooling and evaporative power of the wind on its dry or moist surface at body temperature, and the powerful effects of an open-air life with exposure to wind on the human body have been studied. It seemed of interest, then, experimentally to study the effect of wind on the germination and growth of seedlings. Mustard and cress seeds were grown on lamp-wicks, which were kept moist by their ends dipping in basins of water.

The control seeds, in the relatively still atmosphere, were grown within a glass jar on a damp lamp-wick. The jar was placed horizontally, with one end of the lamp-wick dipping in a saucer of water. The seeds exposed to wind were grown in a glass jar, the bottom of which had been removed, and the neck inserted in the opening of the electrically driven fan, so that air was sucked, or blown, through the jar at the rate of approximately 5 metres a second. The lamp-wick in this case also dipped into a basin of water placed outside the jar. The blower-fan used was one made by Keith Blackman, and was of the type used for ventilating war-ships. It ran day and night with smoothness, and never failed during the whole course of the experiments, which lasted many weeks.

Some of the seeds exposed to wind did not sprout, some just sprouted, and a few just showed two green leaves on the end of a very short sprout, which was bent and horizontal; the contrast with the control being very striking. While the lamp-wick exposed to wind was quite moist, it appeared as if the upper surface of the seeds exposed to wind might not be wet enough for growth.

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* Leonard Hill, 'The Science of Ventilation and Open-Air Treatment.' Spec. Rep. Series. Nos. 32 and 52. Med. Research Council.

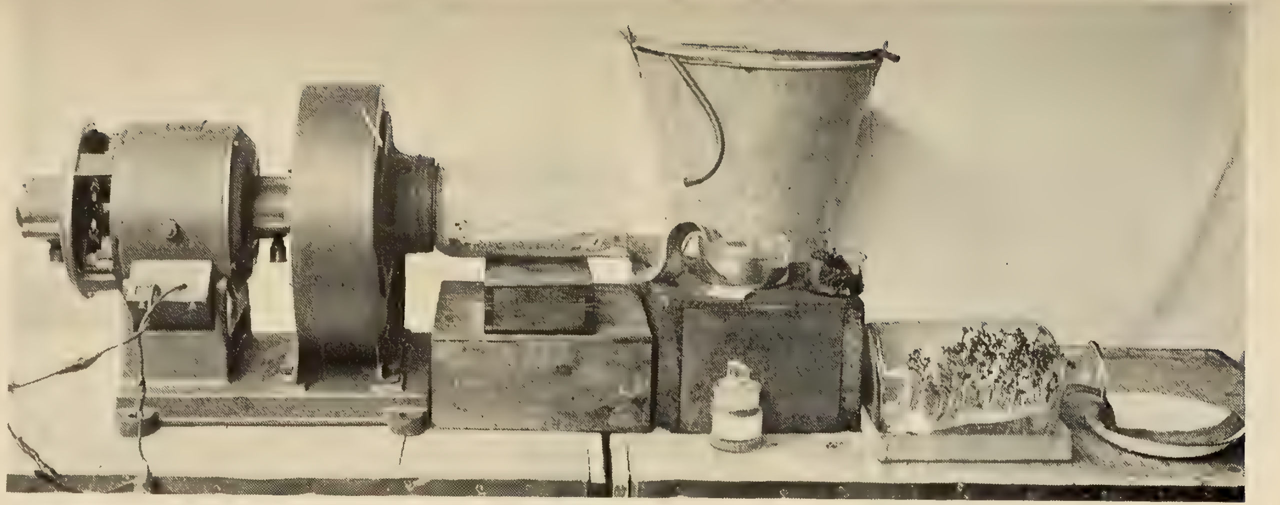


Fig. 1.

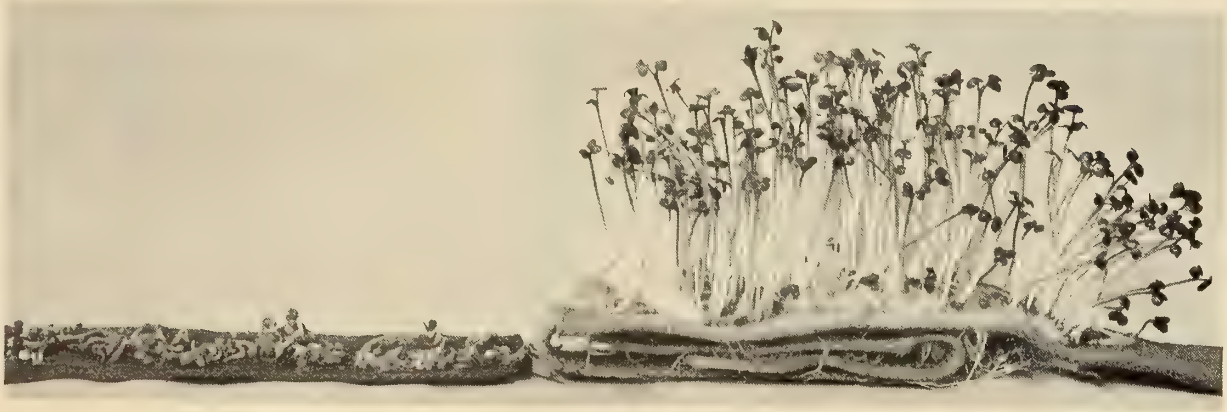
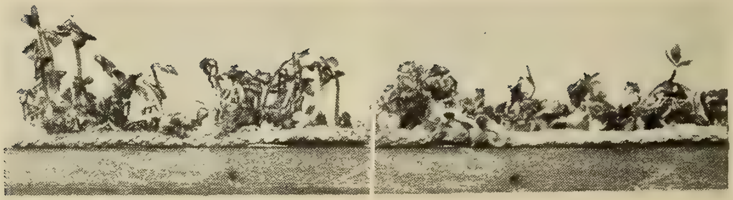


Fig. 2.



A Fig. 3. B

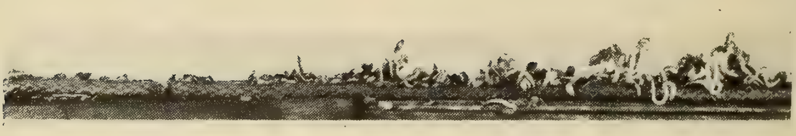
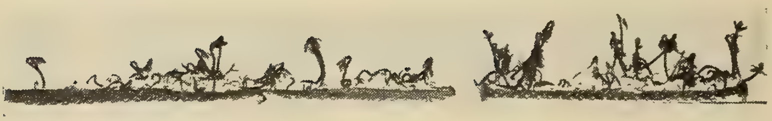


Fig. 4.



B Fig. 5. A

Experiment II was arranged, therefore, as shown in Plate 2. By means of a pail of water, and a syphon tube ending in a glass capillary tube, a gentle trickle of water at room temperature was kept flowing along the wick. Fig. 2 shows that the growth of the seeds exposed to wind was vastly less than the control. The seeds sprouting in the wind were bent close to the wick, and none of them showed root-hairs, which were abundant on the control seedlings.

Experiment III.—Part of the lamp-wick, with some of the control seedlings on it, was transferred to the wind jar for a few days. The seedlings maintained their upright position, did not dry up and wither, but grew very slowly if at all.

Experiments IV, V, and VI.—Experiment II was repeated in warmer surroundings, and the wind which was made to blow through the jar scattered an abundant supply of water spray over the seeds. The results were of the same order, but the growth a little greater. The following determinations were made for me by Mr. A. Webster from random samplings :—

	Still-air seedlings.	Wind-exposed seedlings.
	Mustard.	
Average length of plant	55 mm.	22 mm.
Total solids	8·3 per cent.	21·3 per cent.
Water	91·7 ,,	78·7 ,,
Protein	30·0 ⎫ per cent. of total	26·8 ⎫ per cent. of total
Ash	7·8 ⎭ solids	8·4 ⎭ solids.
	Cress.	
Wet weight of 40 plants	0·7616 grm.	0·5139 grm.
Total solids	8·4 per cent.	13·3 per cent.
Water	91·6 ,,	86·7 ,,
Protein	31·2 ⎫ per cent. of total	27·3 ⎫ per cent. of total
Ash	14·8 ⎭ solids	19·7 ⎭ solids.

The bent, contorted, wind-exposed seedlings have, when straightened out, one-half to one-third the length, and contain more solids, less water, more ash, less protein, and, presumably, more cellulose.

Experiment VII.—A piece of cotton net (ladies' veiling) was placed over the seeds so as to anchor them and prevent the wind and water moving them. The conditions were otherwise the same as in Experiment II. A water film formed over the meshes of the net, and the seeds grew under the shelter of this, raising the whole net as they grew. The growth of the control and the wind exposed under these conditions were much more nearly equal.

Experiment VIII.—The pail of water shown in fig. 1 was heated by a gas-ring placed under it and fed by a tap, so that an outflow of warm water over the seeds was maintained day and night, the whole being arranged conveniently over a sink, so that the waste water ran away. The wind was made to blow out through the jar and scatter the water spray along the seeds. Where the water entered the temperature averaged about 24–27° C. At the end of the wick near the opening of the jar the temperature averaged about 18° C., which was the average temperature of the control. Fig. 3, A, shows the still air, and B the wind-exposed seedlings. The latter shows a growth bent and twisted, but about two-thirds as long, when straightened out, as the control. Analysis showed in this case:—

	Wind-exposed seedlings.	Still-air seedlings.
Average length	20 mm.	30 mm.
Weight of 50 plants	0·6455 grm.	0·968 grm.
Total solids	8·66 per cent.	8·2 per cent.
Water	91·34 „	91·8 „

A difference in size rather than in percentages of water and solid is noticeable. The wind-swept seedlings in all the experiments grew no root hairs, while these were abundant on the controls.

Experiment IX.—Fig. 4 shows the result of growing cress seeds in a hot room kept continuously at 37·5° C. The seeds grown in the wind were kept wet by water which dripped out from a glass tube, the end of the tube lying on the wick close to where the growth is seen to be best. At the left end of the wick the growth is nil, although the wick and the under surface of the seeds were wet. The drying of the upper surface of the seeds was sufficient to stop growth. In the intermediate part the growth was better with the increasing wetness of the seeds. The control was placed so that the water from the wick in the wind tunnel dripped upon it. This dropping rain especially favoured growth in the hot room. It was greater than that shown in Fig. 3, A. Seeds kept just totally immersed did not grow. The amount of moisture the seeds receive is obviously of very great importance.

Experiment X.—Fig. 5 shows the growth in the hot room in wind, B, and out of the wind, A, both wicks being irrigated by falling drops of water so as to make the conditions of water supply as exactly comparable as possible. The growth of the seeds in the wind is still behind that of the control. The approximate average length of five seedlings was 2·5 cm. in the wind and 3·0 cm. in the control. The weight of 26 seedlings minus the seeds was 0·315 grm. in the control and 0·284 grm. in the wind. The percentage of solids was 13·8 and 17·2 per cent. respectively.

The temperature of the wet wick was 29° in the control and 26° in the wind. The evaporative cooling power of the wind may, therefore, have had an effect.

The retardation of growth cannot be attributed to the shaking produced by the motor, because seeds sheltered from the wind, but submitted to the shaking, grew as well as the control seeds.

The conclusion is reached that the stunting effect produced by wind is not only due to a less favourable wetting, but to greater cooling. The growing point may be robbed by wind of heat which is produced in the cellular growth processes—heat which facilitates growth.

I am much indebted to Mr. R. H. Davis, of Messrs. Siebe Gorman, Ltd., who gave me facilities for carrying on this research at a time when the National Institute of Medical Research was being used as a War hospital.

Reflex Times in the South African Clawed Frog.

By W. A. Jolly.

(Communicated by Prof. E. Sharpey Schäfer, F.R.S. Received November 1, 1920.)

(From the Physiology Department, University of Cape Town.)

This investigation was undertaken with the object of determining the times of certain reflexes in a species of frog which has not, so far as I know, already been used for the purpose, viz., the South African clawed frog or toad (*Xenopus lævis*, or an allied species), and by analysing these times to obtain a measure of the delay in transmission of the reflex impulses in the spinal cord, or "synapse time." Spinal and decerebrate animals have been used as well as the intact frog. Einthoven's string galvanometer was employed to indicate the beginning of activity in the limb muscles.

It seemed desirable to eliminate, as far as possible, everything that would tend to introduce doubt into the interpretation of the records, even at the cost of making the experiments more troublesome to carry out. For this reason no strychnine or other drug was used. The spinal and decerebrate preparations were kept alive before experiment until the wounds were entirely healed and the frogs appeared to be in thoroughly good condition. Records were then taken from day to day, and the influence of temperature and of the period of survival after operation was studied. Any operative procedure at the time of experiment was rendered unnecessary by employing the mechanical stimulus of a tap by a slight hammer to the toes, or a squeeze of the toes, and the electrical changes in the limb muscles were recorded by means of the wicks of non-polarisable electrodes, moistened with salt solution and impregnated with kaolin, bound round the limb over the unbroken skin. The wicks were applied at knee and ankle, or thigh and ankle, the skin area covered by each wick being about 1·5 cm. broad. The advantage of this method of leading off is that there is no injury done to the preparation, which can be experimented with for months. A disadvantage is that one cannot record in this way the pure response of a single muscle nor control exactly the points led off from, and the form of the electrical response is found accordingly to be rather variable. The application of a mechanical stimulus to the toes instead of electrical stimulation of a nerve, besides being more "natural," is advantageous from its avoiding injury to the preparation, and also by avoiding the difficulties which arise in recording and interpreting the electrical response when the stimulus is also electrical. Precautions will prevent an induction shock given to a dissected-out nerve from affecting the galvanometer, but it is often difficult to get rid of some slight action upon the string, and the absence of this complication makes me more confident in interpreting the curves. There is also no danger of the stimulus spreading further than is desired. The disadvantage of the use of a tap or squeeze is that a direct stimulation of muscle or nerve might perhaps be caused, whose electrical expression would be visible on the record and be mistaken for a reflex effect. This is a real difficulty in the case of homonymous stimulation, and the attempt has been made to avoid it by stimulating the toes alone and not the rest of the foot. The difficulty does not arise in the case of the heteronymous reflex where the electrodes are applied to the limb opposite to the one stimulated. Here there is no appearance on the records of anything which could be the expression of a direct excitation of the foot.

The photographic records of the galvanometer movements are made upon plates falling behind the slit of Cremer's apparatus at a rate of about 1 metre per second. The shadow of the string of a small string galvanometer with electromagnet is also thrown on the slit and signals the moment of stimulation. A tuning-fork, vibrating at the rate of 200 D.V. per second in front of the slit, throws its shadow on the plate and serves as time marker. The vertical lines showing the direction of the slit are produced by the teeth of an episkotister. In some cases the episkotister has not been used. The curves are measured by the aid of an enlarging camera. The intervals of time are given in thousandths of a second (σ), fractions are also given.

The frog is suspended in a jacket with the head bandaged as a precaution against any stimulation of the acoustic nerves when the mechanism for giving the stimulus is set in action.

Two plates of vulcanite, hinged below with lead to form a V, and held on an adjustable stand, are brought up to the foot to be stimulated so that the toes lie in the opening of the V, and the vulcanite plates are closed on them without pressure. Two forms of stimulation are employed. One is innocuous and consists of a tap from a light hammer, held on a separate stand and attached to a spring. The head of the hammer, which bears a piece of platinum foil, is arranged to strike the vulcanite plate lying against the frog's toes on the release of an electromagnet by the plate-holder as it travels. On the outer surface of the vulcanite is a piece of platinum foil, and the circuit through the signal galvanometer is closed when the hammer-head comes in contact with this.

The other form of stimulus may be termed nocuous, since after several applications it will damage the foot. In this form the hammer is replaced by a spring-vice held on a separate stand, which, on the release of the electromagnet, forcibly compresses the vulcanite arrangement in which the toes lie. This stimulation has been chiefly used in order to obtain the heteronymous reflex in the decerebrate frog.

By the use of these arrangements a mechanical stimulus, weak or strong, can be given without moving the foot perceptibly. The hammer-tap is itself of the nature of a slight sudden squeeze, since the foot is not displaced to one side by the blow, but held against the vulcanite plate. The strong squeeze is given in such a way that the foot remains stationary. Thus, there is no change in the position of the wick on stimulation, and consequently no alterations of resistance from this cause in the galvanometer circuit, nor is there any stretching of the muscles.

The method employed in the experiments is to attach the wicks of the electrodes to one leg and to lead off from it during the course of the experiments. The toes on the same side as the electrodes are first stimulated, then the toes on the opposite side, and so the homonymous and heteronymous reflexes are recorded alternately. Dummy electrode wicks are attached to the opposite leg, to keep the conditions uniform on the two sides.

It is not proposed in this communication to compare in any detailed way the results obtained in the clawed frog with the findings in Rana or in the mammal. I have not at present access to all the literature on the subject.

It is not easy to compare the reflexes obtained from a preparation studied when it is thoroughly "normal" with those observed after the administration of drugs or previous cooling to a low temperature, or which have been subjected to dissection immediately before experiment; nor is it easy to compare the results of mechanical stimulation with those recorded when the stimulus is electrical and may be suspected of spreading, as Miss Buchanan (1) points out, even so far as to affect nerve-fibres in connection with the motor cells or nerve of the opposite side.

A considerable number of observers have employed strychnine to raise the excitability of the cord ; indeed, in the case of Wundt's (2) work the doubt has been expressed as to whether he ever obtained the heteronymous reflex in an undrugged preparation ; but the action of strychnine on the neural mechanisms is so profound that responses and latencies observed under its influence must be studied apart from those of the undrugged preparation.

The range of temperature at which this investigation has been carried out is higher than that generally employed with the frog, and the shortness of the periods of delay obtained are to be explained to some extent on this account.

The Spinal Frog.

The spinal cord was cut aseptically and the wound allowed to heal thoroughly. The reflexes were recorded at intervals while the frog survived. The room temperature was noted, and observations at higher temperatures were also made by keeping the frog during experiment in an incubator which could be regulated to different temperatures. The frog's temperature was then noted from a thermometer in the mouth.

The reflex times are found to vary a good deal in the same frog and between different frogs, and it is advisable to make a considerable number of observations and to average the results.

The factors which may be expected to influence the length of the reflex time are the temperature, the length of the period of survival after operation, and the length of the conducting nervous path, that is to say, the size of the animal. Its age will doubtless also have an influence and can be estimated from the size. Frogs were chosen of about the same size. They were allowed to live as long as possible, and *post mortem* the nerves were dissected out and measured. Towards the end of the period of survival the reflex times appear to lengthen somewhat.

The response in the spinal frog to a tap on the toes is a sudden sharp flexion of the same leg and extension of the opposite leg. There is also adduction of the limbs. The reflex is of a jerky character.

The chief object of the investigation in the spinal preparation was to study the time difference between the onset of homonymous and heteronymous reflex activity of the hind limbs. In the spinal mammal it has been shown that this interval is very brief, amounting only to about 0·001 second. In the spinal frog I have found this difference to amount sometimes to as much as eight thousandths of a second (8 σ), that is to say, the crossed is longer than the same-side time by this interval, but sometimes there is a reversal and the crossed time is shorter than the same-side time. In other cases no appreciable difference of time is found between the two reflexes.

It was hoped that the difference of time would be suitable for an investigation of the influence of temperature upon the delay at a synapse, on the assumption that an additional synapse is interposed in the path of the heteronymous reflex, but it is difficult to explain on this assumption the complete disappearance of the interval in some experiments or the occasional reversal. On the other hand a difference of 8 σ is quite consistent with the view that the heteronymous path involves more synapses than the homonymous and indeed is most easily explained in this way. One is inclined to contemplate the possibility that as the conditions change, the path taken through the spinal cord in the heteronymous reflex may alter.

The following results were obtained by the use of the innocuous stimulus. The times are given in thousandths of a second (σ) and the temperature in degrees Centigrade (see Table on p. 36).

The time difference between the reflexes on the two sides, when present, is small. In the above experiments, which I have grouped together, the longest time difference, 4·1 σ occurred at the lowest temperature used, 14°, but the differences generally do not seem to have a simple relation to the temperature; at any rate, from the data at present available such is not evident. It may be mentioned that frog B, which gave the difference of 4·1 σ, died three days later (forty-five days after operation) so that presumably at the time of experiment it was not in the best condition, and this may have had an influence upon the time difference.

I do not think that the above experiments taken by themselves would justify us in assuming that in the spinal frog at the temperatures employed, and with the frogs in good condition, the intraspinal path of the heteronymous reflex involves a larger number of synapses than that of the homonymous reflex. The fact that the former may sometimes have a shorter time than the latter, which seems also to have been observed by Rosenthal (1), tells against such a view. It would rather appear that we are dealing with mechanisms involving the same number of neurones on the two sides. Even if they are similarly constituted as regards number of synapses one could hardly expect that the mechanisms would work absolutely synchronously. The fact that the homonymous time is usually slightly the shorter may possibly be due to the motor neurones responding rather more readily to stimuli reaching them through afferent nerves from the same side of the body. Doubtless the greater number of the reflex flexions of a leg will, in the ordinary course of

Frog.	No. of experiment.	Days after operation.	T.	Homonymous reflex time.	Heteronymous reflex time.	Difference obtained by subtracting average homonymous from average heteronymous time.
A	1	13	20 (room)	12·0	17·0	
				16·4	12·8	
				15·6	13·5	
				15·8	15·9	
				15·3	15·9	0
B	2	40	20 (gullet)	13·9	13·9	
				14·5	16·4	
				13·5	15·6	1·3
	3	34	28 (room)	14·0	15·5	
				11·7	13·1	
				11·0	13·2	1·7
	4	35	27 (room)	11·1	14·8	
				11·8	12·8	
				12·0	12·0	1·6
	5	35	23 (room)	11·4	10·7	
				14·0	10·2	
				14·4	10·7	− 2·8
	6	34	21 (room)	20·8	20·4	
				16·6	18·0	
				15·8	18·2	1·1
	7	36	20 (room)	18·1	22·1	
				17·0	20·0	
				17·1	19·3	
				18·0	21·6	
				18·0	18·0	
				16·5	21·7	3·0
	8	42	14 (gullet)	24·0	26·4	
				22·1	26·4	
				19·3	25·0	4·1
C	9	14	30 (room)	10·8	11·0	
				9·9	9·7	
				10·1	10·0	− 0·1
	10	13	28 (room)	10·8	13·6	
				10·4	12·4	
				11·1	10·9	1·5
	11	14	20·5 (room)	11·5	13·8	
				12·2	12·8	
				—	12·8	1·3
	12	13	19	10·5	13·8	
				11·8	14·0	
				12·3	15·1	2·8

Average of above heteronymous reflexes (41 in number) .... $15{\cdot}7\sigma$
Average of above homonymous reflexes (40 in number) .... $14{\cdot}3\sigma$

Average difference .... $1{\cdot}4\sigma$

events, be aroused by sensory stimuli playing upon the same limb. This may have led to slightly greater facility and shorter delay in transmission at the same-side synapse.

The actual time of the reflex varies, of course, with temperature, size, and condition of animal.

The temperature in the room in the Physiology Department where the frogs are experimented upon rises in Summer as high as 26° C. If we select a reflex time recorded at a room temperature of 23° C. from a frog in good condition, as for example frog B, Experiment 5, first response, thirty-five days after operation, we find an interval of 11·4 σ for the total reflex time. The length of nerve from toes to cord and from cord to thigh was found *post mortem* in this frog to be 16 cm. The rate of conduction in the nerve of *Xenopus* at 23° C. was determined in the usual manner by electrical stimulation at two points, with the electrical change in the muscle as indicator, and was found to be 37 metres per second. The latency of the muscular response to nerve stimulation close to the muscle was found to be 2·4 σ. If we allow 1 σ for the latency of sensory nerve endings we may suggest the following analysis:—

Total reflex time		11·4σ
Nerve conduction	4·3	
Muscle latency	2·4	
Latency of sensory endings................	1·0	7·7
Synapse time		3·7σ

The conclusion to which one is led by these results is that, at the higher range of temperatures, and when the frog is in good condition, the homonymous and heteronymous reflexes each involve a path through the spinal cord which contains one synapse or set of synapses having a delay of 3·7 σ. The reflex in the spinal frog is of the nature of a jerk, and we may compare this synapse delay of 3·7 σ in *Xenopus* with the delay of about 2 σ found for the knee jerk in the mammal (3), (4).

There is however evidence that at lower temperatures, or when the frog is not in good condition, the short path to the crossed motor neurones, which we have described as containing one synapse, is not open. I have grouped together the remainder of the experiments performed on the spinal frog as examples of this. The figures are as in Table on p. 38.

In this second group of experiments there is a considerable time elapsing between the beginning of activity in the two limbs. The extra delay of the crossed reflex amounts to about 8 σ, which suggests that a heteronymous path is being traversed here which contains more synapses than the homonymous path. We have seen reason to conclude that the delay at one synapse is almost 4 σ, and we have to consider whether this difference of

Frog.	No. of experiment.	Days after operation.	T.	Homonymous reflex time.	Heteronymous reflex time.	Difference obtained by subtracting average homonymous from average heteronymous time.
C	13	17	16·5 (room)	15·0	23·1	
				15·2	21·3	
				12·9	26·5	
				11·8	22·8	
				13·5	17·6	
				13·7	—	
				15·5	—	
				15·6	—	
				14·7	—	8
D	14	3 (after cord section) 123 (after decerebration)	19 (room)	14·4	18·1	
				14·4	22·5	
				21·5	21·1	
				15·4	26·6	
				—	32·6	7·8
E	15	20 (after cord section) 141 (after decerebration)	30 (room)	8·4	16·2	7·8
A	16	2	15·5 (room)	18·9	23·6	
				19·1	22·2	
				18·9	21·8	
				16·2	28·8	
				17·0	26·5	
				18·3	37·0	
				13·2	22·1	
				19·0	18·2	
				19·7	—	7·2
B	17	1	15·5 (room)	12·2	25·8	
				12·7	24·8	
				15·5	29·0	
				18·4	23·8	
				17·8	22·2	
				—	17·4	8·5

Average of above heteronymous reflexes (25 in number) .... 23·7σ
Average of above homonymous reflexes (28 in number) .... 15·7

Average difference .... 8·0σ

about 8 σ can be accounted for by the interposition in these cases of one extra synapse in the crossed path, whose delay has been increased to as much as double, owing to lower temperature or less satisfactory general condition of the preparation. The average homonymous time is practically the same in the two groups of experiments.

I find it difficult to believe in so great a lengthening of the delay at one synapse, and think it more probable that two extra synapses have been interposed in the crossed path in this group of experiments, and conclude that the heteronymous path employed here contains two synapses more than the homonymous, or three synapses in all.

It seems to depend upon the general condition of the frog as to whether the short path is pervious or not. Thus in Experiments 16 and 17, where the observations were made on the second and first days respectively after section of the cord, and where the frogs may not entirely have recovered from the hæmorrhage and other immediate effects of the operation, and where the wounds were not yet healed, the short path is apparently impervious. In Experiments 14 and 15, where the frogs had been decerebrated and preserved in the laboratory for several months before the cord was cut, and where they were emaciated, with flabby muscles, the long path appears to have been used. Even the high temperature used in Experiment 15 does not diminish the extra delay of the crossed reflex, although it reduces the time of each reflex.

In diagram (fig. 1) the time differences in all the experiments are arranged in order of magnitude, and a step-like appearance is shown.

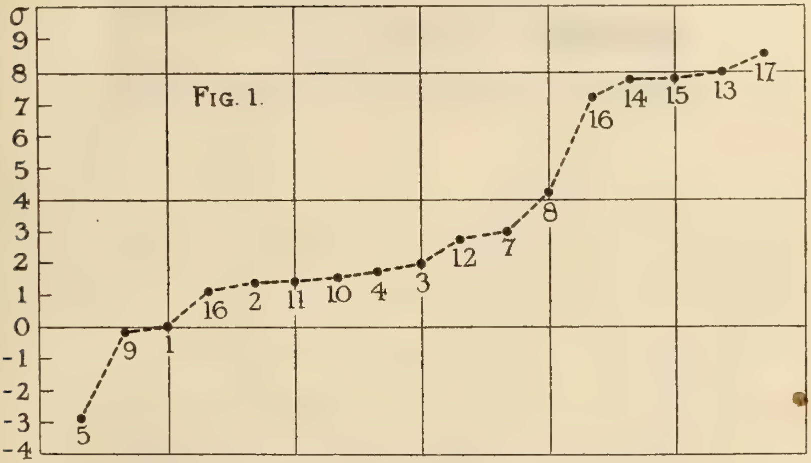


Fig. 1.—Diagram giving in order of magnitude the time differences obtained by subtracting the homonymous reflex times in the hind-limb of the spinal frog from the heteronymous reflex times. The numbers are the numbers of the experiments and the time differences are plotted in thousandths of a second (σ). The diagram exhibits a step-like character. A negative difference means that the heteronymous reflex time is shorter than the homonymous.

The Form of the Electrical Response in the Spinal Frog.

Figs. 2 and 3 represent forms of variation recorded on homonymous stimulation of the toes, fig. 4 on heteronymous stimulation. The electrical variation at its commencement resembles that of a twitch, but the curve shows, after the initial deflections, other oscillations which are absent from the record of a twitch: thus, for example, in fig. 4 there is a series of three

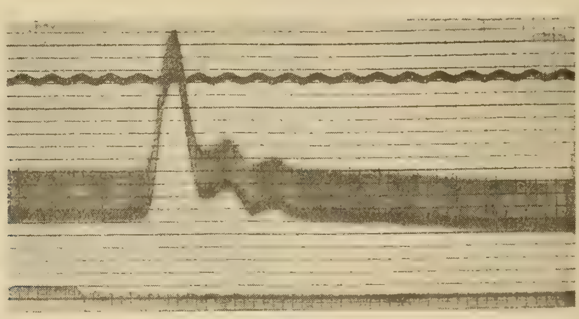


Fig. 2.

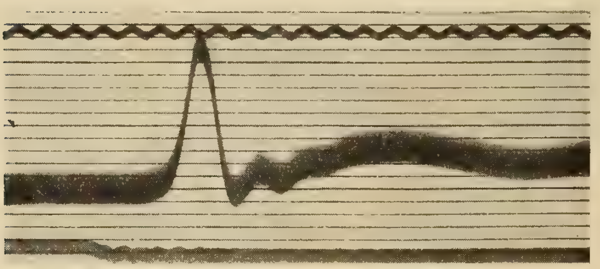


Fig. 3.

Figs. 2 and 3.—Electrical variations evoked reflexly from the muscles of the hind-limb of the spinal frog by mechanical stimulation (hammer tap) of the same-side toes. The wicks of non-polarisable electrodes are applied over the skin at knee and ankle. The upper line is the shadow of a tuning-fork vibrating at the rate of 200 D.V. per second. The middle line is the shadow of the string of the galvanometer. The lower line is the signal, giving the moment of tapping the toes. The curves are to be read from left to right. All are somewhat reduced in size.

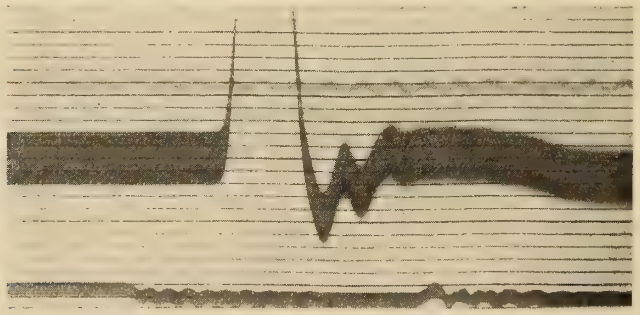


Fig. 4.—Electrical variation evoked reflexly from the muscles of the hind-limb of the spinal frog by tapping the heteronymous toes. Time marker and signal as in figs. 2 and 3.

small oscillations occurring at a rate of about 216 per second. This record was made at a temperature of 18° C. Some curves show a double summit, as though one twitch were followed closely by another. These resemble some of the curves figured by Miss Buchanan (1). Sometimes the records are complicated in such a way as to suggest that the reflex is composed of a series of twitches. Occasionally, there is visible in the records of the homonymous effect a small initial deflection, following the stimulus after an interval of a few σ. It was at first thought that this might be some fortuitous effect upon the string due to the mechanism of stimulation, which involves the release of an electromagnet, but precautions do not prevent its occasional appearance, and it must be attributed to some form of direct stimulation of the foot by the hammer tap. Its very short latency is consistent with this.

The twitch-like nature of the response is what one would expect from the jerky movement of the muscles in the spinal preparation. The succeeding series of small rapid oscillations indicates that there is also an element of tonic contraction involved.

The Decerebrate Frog.

The cerebral hemispheres were removed aseptically and the wounds allowed to heal. The frogs were preserved until they died, usually several months, and the reflex times were tested at intervals. The wicks of the non-polarisable electrodes were applied over the skin, either at thigh and ankle or knee and ankle. The reflex movement elicited by stimulating the toes in the decerebrate animal has not the twitch-like character which it assumes in the spinal frog. The reflexes are not obtained with the same certainty as in the spinal frog. The heteronymous reflex sometimes fails even when the stimulus used is the nocuous form of a strong squeeze. In other cases the reflex is delayed very considerably. Under these circumstances, it seemed desirable to make a number of alternated observations of crossed and same-side reflex activity at intervals of a day or two, and to average the results, in order to obtain a measure of the time difference between the commencement of the two reflexes, rather than to rely upon the differences found in individual pairs of responses.

With regard to the delayed heteronymous responses, such times as the following have been recorded: 43·1 σ at 22°, thirty-eight days after decerebration; 45·4 σ at 26°, twenty-nine days after decerebration; and 55·2 σ at 19·6°, ninety days after decerebration. Such long delays have not been found in spinal preparations, but in the intact frog I have found periods of 52 σ, 57·5 σ, and 59 σ, and also some of a still greater duration, viz., 82·5 σ, and 92·5 σ. Waller (5) records delays for the crossed reflex in Rana as long as 228 σ.

The absence of prolonged periods of delay in the spinal frog, and their presence in the decerebrate and intact animals, suggest that in these cases the simple spinal reflex has failed, and that we are dealing with responses which involve the lower part of the encephalon. In favour of this view is the fact that in some records we find, in addition to the deflection which obviously represents the spinal crossed reflex, a second deflection, appearing much later in the curve, which seems to be due to a second outflow of impulses from the centres. Thus, in frog E, fifty-two days after decerebration, we have at 23° a deflection occurring 15·8 σ after stimulation, and a second 44 σ after stimulation, and in the same frog, eighty days after operation at 21·5°, a deflection at 15·3 σ and a later one at 41·2 σ. If this view is correct, then these delayed responses should be placed in a class by themselves, and not averaged along with the other responses.

The following are the average times in thousandths of a second of the heteronymous reflex which have been obtained from decerebrate frogs by the use of the nocuous stimulus at an average room temperature of 21·6° C.: frog A, 19·4 σ; frog B, 15·5 σ; frog C, 22 σ; frog D, 16·8 σ; frog E, 17·1 σ; frog F, 16·2 σ; and frog G, 17 σ. The individual times are arranged in diagram (fig. 5) in order of magnitude.

Fig. 5.

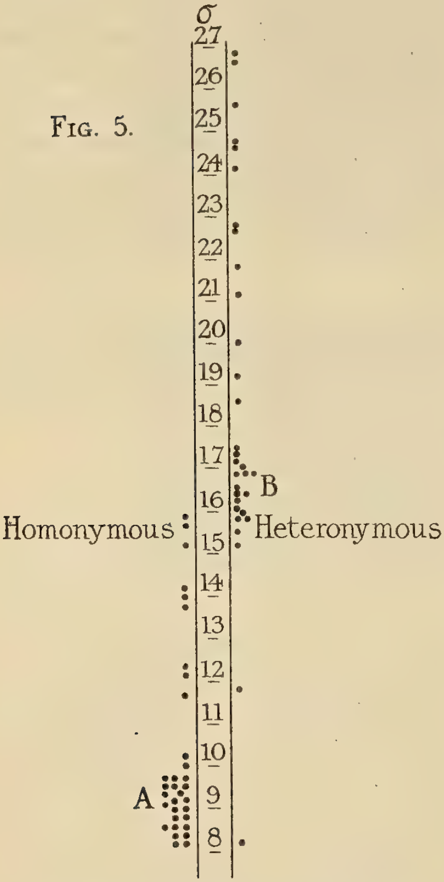


Fig. 5.—Diagram giving the reflex times obtained from the hind-limb of the decerebrate frog on homonymous and heteronymous stimulation of the toes. The times are plotted in thousandths of a second (σ). The diagram shows two groups of reflex times, a group, A, of homonymous reflexes averaging 9σ and a group, B, of heteronymous reflexes averaging 16·4σ.

The average of all these crossed reflex times—thirty-five in number—is 18·5 σ. Two of them, both recorded from frog E, are exceptional. In the one case, the deflection appears 8·3 σ after stimulation; in the other case, the onset of the deflection occurs 11·8 σ after stimulation, and is followed by a large second deflection 4 σ later, *i.e.*, 15·8 σ after the stimulus. This time —15·8 σ—agrees with other crossed reflex times from the same and other decerebrate frogs, but 11·8 σ is exceptionally short for a crossed reflex, and agrees rather with the homonymous reflex times. There have been a number of other cases recorded where the commencement of the deflection shows a double character, a second deflection following the first by about 4 σ. Caution must be observed in the interpretation of such curves recorded by means of electrodes on the skin, where one has not point leads on individual muscles. It is easy to understand how complicated curves can be obtained from a single muscle when an electrode is not applied exactly to the point where the motor nerve enters the muscle. In the cases mentioned, however, the form of the curve, taken together with the unusually short delay, rather suggests that, for some reason, the impulse, in passing from one side of the cord to the other, has here taken a short path which is not usually open in the decerebrate frog, and that another impulse, traversing a path longer by the addition of one synapse, has later caused a second discharge from the motor neurones. It may be that the short path used here in the decerebrate preparation, as a rare exception, may be the same path as is usually followed in the spinal preparation, where, as we have seen, the heteronymous delays are, with frogs in good condition, similar to the homonymous.

If we attempt to analyse the average delay of the heteronymous reflex in the decerebrate frog, assuming the delay at a synapse to be the same as in the spinal frog, the following may be suggested :—

Average delay of heteronymous reflex (35 records)	18·5σ
Conduction in nerve (16 cm. at 35·5 metres per sec., 21·6° C.)	4·5
Muscle latency	2·4
Latency of sensory endings	0·5
Delay at three synapses (3·7σ each)	11·1
	18·5σ

It is doubtful whether any allowance need be made for the latency of sensory endings. With the strong squeeze it is probable that the afferent nerve fibres may themselves be stimulated.

Homonymous Responses in the Decerebrate Frog.

The times of the homonymous responses determined in the decerebrate frog are arranged in order of magnitude in diagram (fig. 5). The average delay of all the responses, thirty-three in number, is 10·7 σ. The average temperature is 21° C.

It will be seen that a number of records have been obtained of what appear to be reflex effects from the same-side limb, which have a very short delay. It is evident from diagram (fig. 5) that there is a group of same-side responses averaging 9 σ, and a group of crossed responses averaging 16·4 σ. If these homonymous responses are in reality reflex then the time difference between homonymous and heteronymous reflexes is 7·4 σ. This is just double the figure which we have found in the spinal frog to represent the delay at one synapse, and we would conclude from this that the path of the impulse across the spinal cord involves two synapses more than the homonymous path, or three synapses in all, with a latency of 3·7 σ at each synapse.

As regards the brief delay, it is certainly surprising to find such short reflex times as 9 σ. At first one was inclined to think that they must be due to direct stimulation as opposed to reflex action, and the strong squeeze is undoubtedly capable of causing direct stimulation at times, but the latency with direct stimulation is much shorter than 9 σ. The following experiments show clearly the difference in latency between the muscular response to stimulation of a motor nerve in these decerebrate frogs and the group of responses which we are considering. The sciatic nerve was isolated at the back of the thigh, without being cut, and small vulcanite rods were placed above and below the nerve. The nerve was then mechanically stimulated by a light hammer tap on the upper rod, the signal circuit being closed at the same moment. The wicks of the non-polarisable electrodes were attached round uninjured skin, one below the knee, the other at the ankle. The latency of the muscular activity at 20° C. was found to be 3·2 σ, after deducting 0·7 σ for nerve conduction at the rate of 34 metres per second through 2·3 cm. of nerve intervening between the point stimulated and the gastrocnemius muscle. In another decerebrate frog, twenty-one days after operation, the latent period of the muscle was found by the same method to be 4 σ at 17° C. With electrical stimulation of the nerve by an induction shock the latent period has been found to be as short as 2·40 σ at 22° C.

Since it is found in this way that the latent period of the muscles of the decerebrate frog is so short, it is difficult to entertain the view that delays of over 8 σ, obtained by squeezing the toes in lively decerebrate preparations in good condition, with the circulation intact and no operative interference at the time of experiment, can be other than reflex effects. Under the conditions of experiment one would not expect the muscle latency to be lengthened to such an extent, and with the data at present available we must regard this delay of 9 σ at 21° as a reflex time, which includes the delay at one synapse.

It must be borne in view that the decerebrate preparation suspended as in the experiments is not very quiescent. Alternating with periods of quiescence are periods of violent "struggling" movements. Presumably afferent impulses initiated by the stretching are being received by the centres from the sensory receptors of the limbs which are hanging extended under the influence of gravitation, and these impulses cause the motor cells to discharge at intervals. There will thus be periods of gradual increase in the excitability of the motor centres ending in discharge. Perhaps the shortest times recorded are due to mechanical stimuli which chance to be imparted at a time when the motor centres are nearing their maximal excitability, and are to be regarded as examples of what may be termed "hair-trigger" action.

The Reflex Nexus between Forelimb and Hindlimb.

Some experiments were performed in which the reflex activity of the hindlimb was recorded in response to a mechanical stimulus applied in the one case to the hand of the same side, and in the other to the hand of the opposite side. The electrodes were applied at knee and ankle and the hand was subjected to the strong squeeze. On stimulation of the hand of the same side the following delays were obtained from three decerebrate frogs at room temperature of 22° C.:—(1) 16·4 σ, sixty-eight days after operation; (2) 16·9 σ, seventy-six days after operation; (3) 16·7 σ, thirty-eight days after operation; (4) 23·9 σ, thirty-eight days after operation; and (5) 11·8 σ, forty-eight days after operation, giving an average delay of 17·1 σ. On stimulating the hand of the opposite side the following delays were recorded from two frogs at room temperature of 22° C.:—(1) 17·5 σ, forty-eight days after operation and (2) 15 σ, thirty-eight days after operation.

From these figures one cannot assume a difference in time between the cases where impulse descends on the same side of the cord from the hand stimulate, and where it has to cross in descending to the opposite side. Further, the times recorded agree well with those obtained from a hind-limb on stimulating the opposite foot. The length of path to be ascribed to nerve conduction from hand to spinal centre is found on dissection to be shorter than that from foot to spinal centre by about 5 cm., so that we deduct 1·4 σ from the average time of the latter reflex in order to compare with the former, and obtain the same figure.

We conclude that the reflex path from fore-limb to hind-limb, either homonymous or heteronymous, contains the same number of synapses as the path from foot to opposite hind-limb, viz.: three synapses with a delay at each of about 3·7 σ.

We may compare this path with that for the scratch reflex in the spinal mammal as given by Sherrington (6). This author regards the reflex path when the skin of the shoulder is stimulated as consisting of three neurones, and as entering the grey matter twice, that is to say, it is a disynaptic arc. But Sherrington states that this does not mean that there are necessarily only two synapses, but that there may be other synapses due to the interposition of one or more short intraspinal neurones. The diagram given by this author would be consistent with the results here obtained for the flexion reflex in the frog on stimulation of the hand, if we assume that a short intraspinal neurone occurs between the long descending propriospinal neurone and the motor neurone, and that this intraspinal neurone connects not only with the motor neurone on one side of the cord but, by a branch, with that of the other side also. The chain would thus consist of four neurones with three synapses.

A number of delayed responses were obtained from the hind-limb on hand stimulation, similar in time to those recorded from stimulation of the foot. Thus homonymous hand stimulation gave a delay of 42·5 σ, and heteronymous stimulation delays of 57·5 σ, 70 σ, and 72 σ.

In one experiment where the stimulation was given to the homonymous hand a reflex from the leg was recorded 16·9 σ after stimulation and another deflection followed 58 σ after stimulation. Experiments such as these suggest that the delayed reflexes involve higher centres than those of the spinal cord, and that the path through these centres in the lower part of the encephalon involves a large number of synapses. In a double response the segmental spinal centre has discharged with its brief delay, while the afferent impulse has also ascended to the brain and has led to a second reflex with a long delay. If we assume the same delay at a synapse in the encephalon as we have seen reason to believe affects the spinal synapse, then in the experiment last mentioned the long path through the brain would contain ten or eleven more synapses than the spinal path.

We have assumed that the heteronymous hind-limb path, and also the fore-limb to hind-limb path, contain three synapses, and we have been led to this conclusion by using for comparison the reflex in the spinal frog with its single synapse and the group of decerebrate reponses marked Group A on diagram (fig. 5), which we assume to be reflex in nature and to involve one synapse. The possibility, however, is not excluded that homonymous reflexes in the decerebrate preparation may, in response to some forms of stimulation, employ a path involving two synapses. It must be borne in mind that the stimulus used to evoke reflexes in the decerebrate frog has been the powerful squeeze. This is desirable in the case of the crossed reflex, because it requires a strong mechanical stimulus to elicit the crossed reflex with fair regularity, but the homonymous reflex is more easily obtained. The light hammer-tap or innocuous stimulus is sufficient to produce it, and there is some evidence that the central delay is then longer than with the stronger form of stimulus. My observations have not yet extended far in this direction, as attention was concentrated in the case of the decerebrate frog on the effort to obtain crossed reflexes with constancy, and to compare their time with the homonymous reflexes elicited by the same form of stimulation. This necessitates a strong squeeze. It would be advisable in future work to compare the crossed reflex, evoked as here described, with the same-side response to the innocuous stimulus. Even with the data before us, it may be possible to recognise a form of homonymous reflex whose path involves two synapses. If we disregard Group A in diagram (fig. 5) altogether for the present, and average the remainder of the homonymous reflexes, we have 14·4 σ for the homonymous reflex as against 18·5 σ for the average of all the heteronymous reflexes, and 14·4 σ on analysis suggests delay at two synapses, and the extra delay of the crossed reflex would then be attributable to the interposition of one additional synapse. This would accord with previous work on Rana. Thus Wundt (2) finds the extra time of the crossed reflex to be 4 σ, and Miss Buchanan (1) assumes that one extra synapse is interposed in the path of the crossed reflex, whose delay is normally between 10 σ and 20 σ, but may be reduced under the influence of strychnine to 4 σ. This author, however, assumes that there is only one synapse in the path of the same-side reflex with a delay of between 10 σ and 20 σ. We may compare the delay at two synapses in the clawed frog, say 7·4 σ, with the synapse delay in the mammalian homonymous reflex, which has been shown to be 4·3 σ (3), or, according to Forbes and Gregg (7), even less. These authors find the reduced reflex time in the flexion reflex of the cat to lie in general between 3 σ and 5 σ.

Form of the Response in the Decerebrate Frog.

Waves of different rates are observed in the electrical responses recorded. Sometimes the record shows the presence of waves occurring before the stimulus has been given, and these are presumably the electrical expression of the tonic activity of the limb muscles, and when they are very distinct indicate a condition of heightened tonus. Fig. 6 is an example. Here the waves occur at the rate of 54 per second, at a room temperature of 19° C. Other rates recorded are 35 at 16°, 54 and 55·5 at 21·3°, 49 at 22°, and 53 at 25°. These waves are of rather a higher rate than those described by Piper (8) in Rana, doubtless owing, to some extent, to the higher summer temperatures in South Africa. Piper states that on throwing the quadriceps femoris of the frog reflexly into contraction, one obtains an inconstant rhythm, but at summer temperatures the rhythm is one of at least 30 per second. This author considers that these waves are of central origin.

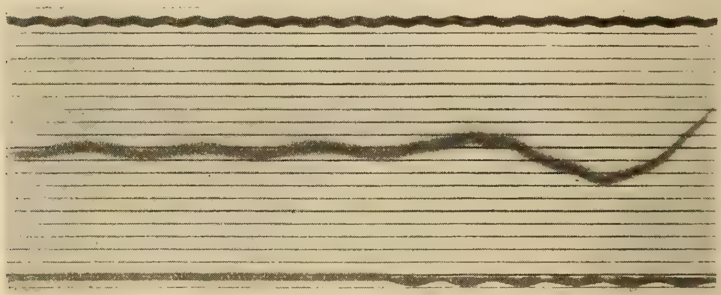


Fig. 6.—Electrical variation evoked reflexly from the muscles of the hind-limb of the decerebrate frog by mechanical stimulation of the homonymous toes. The curve shows waves occurring before stimulation at a rate of 54 per second. Room temperature, 19° C. Time marker and signal as in figs. 2 and 3.

The reflex responses to stimulation exhibit, in addition to waves of similar rate to the foregoing, other waves of greater rate, which occur especially at the commencement of the electrical charge. Fig. 7 is an example of this. The initial waves have here a rate of 206 per second, at a room temperature of 20°. Other rates observed are 277 at 21·8°, 145 at 23°, 137 at 16°, and 128 at 21°. We are reminded of the two varieties of oscillations described by Miss Buchanan (9) in strychnine tetanus as waves and wavelets, but the rapid initial oscillations shown here are of much greater rate than these wavelets, which occur at a rate of 40 to 100 per second, and which Miss Buchanan regards as of peripheral muscular origin. A muscular rhythm so high as 277 per second has, so far as I know, never been suggested, even for the muscles of warm-blooded animals, and we must look for some explanation other than peripheral muscular rhythm. The wicks of the electrodes are placed in these experiments round the thigh and knee, so that the action currents of various muscles which take part in the flexion reflex can show themselves in the electrical response.

I have been struck, in studying the oscillations of the higher rate which appear in the reflex response of the decerebrate frog, with the similarity of the intervals between the deflections and the period which we have seen reason to believe represents the delay at a synapse. Thus, in Experiment fig. 7, the intervals between the rapid deflections at the beginning of the curve are 4·3 σ and 4 σ. In another experiment the interval is 3·6 σ, while the average of the periods in the other cases above mentioned is 7·3 σ, the double of the delay at a synapse. If this is more than a coincidence, it would suggest that these initial deflections of rapid rate are central in origin, and either that they represent the muscular responses to successive discharges from motor neurones, or that one group after another of motor neurones has been thrown into activity, the delay between the excitations of the groups indicating that one synapse or two synapses respectively have to be traversed in the course of this irradiation. As Sherrington (6) points out, grading of intensity of reflex contraction within one and the same muscle group, and even within one and the same individual muscle, seems not only possible but probable, by the numerical implication of more or fewer motor cells, and it seems not improbable that the rapid succession of deflections described above is evidence of the successive implication of neurones in the spinal centres.

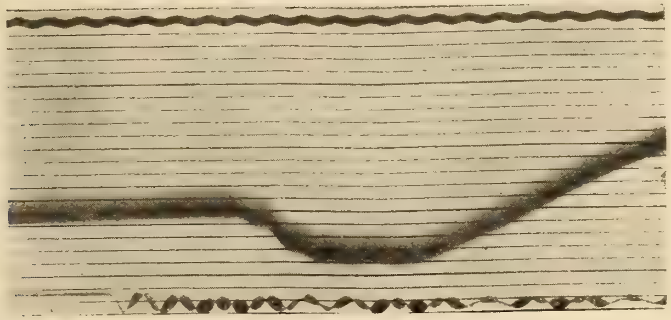


Fig. 7.—Electrical variation evoked reflexly from the muscles of the hind-limb of the decerebrate frog by mechanical stimulation of the heteronymous toes. The response, at its commencement, shows waves at a rate of 206 per second. Room temperature, 20° C. Time marker and signal as in figs. 2 and 3.

Conclusions.

The reflex times of the homonymous and heteronymous reflexes in the hind limbs of the spinal clawed frog have been measured at temperatures ranging from 14° C. to 30° C. The average heteronymous times (sixty-six observations) is 18·7 σ (0·0187 second). The average homonymous (sixty-eight observations) is 14·9 σ. That is to say, the crossed reflex time is longer than the same-side reflex time by 3·8 σ.

It is suggested that the experiments can be divided into two classes, the chief determining factor being the general condition or "fitness" of the spinal preparation. In the first class, where the preparation is normal and the temperature fairly high, the average heteronymous time is 15·7 σ and the average homonymous 14·3 σ. Since the difference between the crossed and same-side reflex times is here very brief, viz., 1·4 σ, and as in some experiments the crossed reflex time is the shorter, it is concluded that the intraspinal paths of the two reflexes are similarly constituted as regards number of synapses, that is to say, each path normally involves one synapse.

In the second class, where the frog is not in the best condition, either on account of a recent operation or from long survival since operation, the short path is not open for the crossed reflex, and a longer path, containing two additional synapses, or three synapses in all, is employed. The average heteronymous time is here 23·7 σ, and the average homonymous 15·7 σ, giving an excess delay for the crossed reflex of 8 σ.

The delay at a synapse in the spinal cord is found to be about 3·7 σ. This is considered to be referable to a single synapse, but it is not intended to exclude the possibility that it represents the delay at a set of synapses.

The electrical variation recorded from the limb muscles is similar to that of a twitch followed by a series of rapid oscillations.

In the decerebrate and in the intact frog, a group of delayed crossed responses from the hind limb are described, having delays ranging from 43 σ to 92 σ. It is suggested that these involve the action of the lower parts of the brain.

Spinal reflexes are recorded from the decerebrate frog, the average time of the heteronymous reflex being 18·5 σ, and of the homonymous 10·7 σ, giving an excess delay for the crossed reflex of 7·8 σ. There is evidence that in some same-side reflexes the delay is longer than the average mentioned, being 14·4 σ, and the excess delay of the crossed reflex is then about 4 σ.

It is considered that, normally, with a strong mechanical stimulus, the intraspinal path of the homonymous reflex in the decerebrate frog involves one synapse (although in some cases it may involve two), while the heteronymous path contains three synapses.

The path followed in the reflex activity of the hind limb, evoked by stimulation of the fore limb, both homonymous and heteronymous, contains three synapses.

The electrical variation recorded from the hind limb muscles of the decerebrate frog shows a series of oscillations at its commencement, at rates reaching 277 per second. It is suggested that these are the expression of irradiation within the spinal centres.

I have to express my indebtedness to Dr. C. Lawrence Herman for his kindness in preparing a considerable number of frogs by decerebration and cord section for use in the investigation, and to my students, Mr. C. A. Oosthuizen and Mr. H. Zwarenstein, for assistance from time to time in the experiments.

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Studies of Photo-synthesis in Marine Algæ.—1. *Fixation of Carbon and Nitrogen from Inorganic Sources in Sea Water.* 2. *Increase of Alkalinity of Sea Water as a Measure of Photo-synthesis.*

By Benjamin Moore, F.R.S., Edward Whitley, M.A., and T. Arthur Webster.

(Original MS. received February 27,—Received in revised form December 2, 1920.)

(From the Marine Biological Station, Port Erin, Isle of Man, and the Department of Applied Physiology, Medical Research Council.)

The series of experiments recorded in this communication were carried out at Port Erin; the subsequent analyses for amounts of nitrogen fixed were made at the temporary laboratory of the Department of Applied Physiology, M.R.C., at the Lister Institute.

The results of the series confirm and amplify those obtained with fresh-water algæ,[*] which showed a convincing uptake of nitrogen from the air, but on account of the change of the medium of growth from fresh to sea water, there are several important modifications in the medium itself as well as in the growing algæ, which appear to us to possess considerable importance in the annual life of the sea, and in the inductance at certain definite periods of the year of increased processes of cell-division and reproduction of species, and possibly in guiding the development of variations in species, and the process of evolution. The details of seasonal variation in growth resulting from intensity of illumination will be given in a subsequent paper; here will be considered the changes in the algæ and the sea water due to the action of light apart from seasonal variations.

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[*] Moore and Webster, 'Roy. Soc. Proc.,' B, vol. 91, p. 201 (1920).

There is in the case of marine plants none of that uncertainty which obtains in the case of the higher terrestrial plants as to how much of the nitrogen being built into organic compounds comes from the roots. Even in the case of such massive plants as Laminaria and Fucus it is obvious that the roots are merely modes of attachment to the rocks, and that the whole plant is built up from the sea water. It is an impossibility that nitrogen could be extracted from the hard stones to which the plants are anchored, and in the case of the floating diatoms, and other minute green cells, which form the phyto-plankton, floating free in the sea, it is clear that the whole organism is formed from the sea water. Hence the entire plant life of the sea is produced by the action of sunlight upon the water of the sea and its dissolved constituents. In so far as sources of ready formed and easily absorbable nitrogenous compounds are concerned, the sea water is remarkably poor, and the volumes of sea water necessary to feed the algæ with nitrogen, were this the source, would be immense. Thus, Gebbing* found the amount of nitrogen as ammonia present in sea water to be only 0·05 mgrm. per litre, and the amount as nitrite plus nitrate as 0·47 mgrm. per litre.† These results are confirmed by some to be given later in this paper.

This paucity of nitrogenous compounds in sea water, while it indicates that the nitrogen of the plant tissues is probably derived from the dissolved elemental nitrogen of the atmosphere, is not however clear proof, for the sea water, for example, contains but a trace of that silica from which the skeletons of the diatoms are derived. It might be argued that in the restless movement of the sea the volume of water which daily laved the plants was ample to compensate for the small amount of dissolved nitrogenous compounds in the water. To settle this query it is obviously necessary to grow marine algæ in a limited volume of sea water and then to determine the amount of nitrogen fixed. If this latter many times exceeds the amounts of nitrogen present as ammonia, nitrites, and nitrates in the sea water used, then clearly, for here there is no soil to obscure the issue, this fixed nitrogen must come from the dissolved nitrogen in the sea water, which in turn came from the nitrogen in the air.

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* J. Gebbing, 'Centralb. f. Bacteriol., 2. Abth.,' vol. 31.

† *Cf.* also Moore, Edie, Whitley, and Dakin, "The Nutrition and Metabolism of Marine Animals," 'Biochem. Journ.,' vol. 6, p. 255 (1912); Raben, 'Wissensch. Meeresuntersuch., Kiel,' vol. 8 (1905).

This has been done in the experiments recorded below, with the clearest proof that marine algæ do so fix their nitrogen.

As photo-synthesis proceeds, and the supply of carbon is drawn from the dissolved bicarbonates, the reaction of the nutrient medium becomes all the time more alkaline.* The increased alkalinity can be used as a measure of the photo-synthetic activity, and was so employed by Moore, Prideaux, and Herdman† in determinations made at Port Erin during the years 1912–1915, and subsequently by Osterhout and Haas.‡ It is interesting to note the level to which this enhanced alkalinity can attain without destroying the green cell which is producing it. Angelstein§ found that, in solutions containing one part of bicarbonate to two of carbonate, the plants continued to give off oxygen. Osterhout and Haas determined by the titration method that the alkalinity can be increased until it reaches a level represented by $P_H = 9$, Moore, Prideaux, and Herdman having previously fixed the limit at P_H—less than 9·1, the last-quoted observers further pointing out that this corresponds to the point at which all the bicarbonates have been converted into carbonates, and this has again been confirmed in the present experiments.

If a sample of normal sea water be titrated with centinormal acid in the presence of a stable indicator such as "di-methyl" or "methyl-orange," a figure defining the entire content of alkali present as bicarbonate is obtained. This figure scarcely ever varies with season or otherwise, and amounts to about 24 c.c. of centinormal alkali per 100 c.c. of sea water. If, now, some sea water be taken, and a green algal growth be exposed in it to bright daylight or sunshine, so as to produce as intense a degree of photo-synthesis as possible, and then the amounts of normal carbonates formed from bicarbonates, as carbon is synthesised into organic compounds, be determined by titrating the alkalinity to an indicator such as phenolphthalëin, it will be found that the limit is just about half the preceding value, namely, 12 c.c. of centinormal alkali per 100 c.c. of sea water. This limit marks the point at which all the bicarbonates of magnesium and calcium present in the sea water have become converted into normal carbonates. If photo-synthesis passed this point, free hydrates of magnesium and calcium would commence to be present in the sea water, and there would be a correspondingly rapid increase in hydroxyl-ion concentration and fall in hydrogen-ion concentration.

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* See Czapek, 'Bio-chemie der Pflanzen,' 2nd edition, vol. 1, pp. 518, 519, where numerous references may be found.

† "Report on the Lancashire Sea-Fisheries Scientific Investigations for 1914," Herdman, 'Trans. Biological Society of Liverpool,' vol. 29, p. 171 (1915).

‡ "On the Dynamics of Photo-synthesis," 'Jnl. of General Physiology,' vol. 1, p. 1 (1918).

§ 'Cohn Beiträge,' vol. 10 (1911).

It is this accumulation of alkali which limits photo-synthesis by killing the cell, for up to quite close to this "all-carbonate" point the cell flourishes and synthesises rapidly, but if kept for some time by too violent exposure to sunlight at this point, the algæ turn yellow and the growth is killed, for there is no recovery, even if removed from the strong light. The degree of alkalinity reached by the green cell before death is, however, remarkable. It is much greater than anything which can be borne by any mammalian cell, and even by any delicate marine animal organism, such as a developing embryo plaice or a fertilised echinus egg.

The increase of hydroxyl-ion concentration and corresponding decrease in hydrogen-ion concentration are such that the water shows a full pink with phenolphthalëin, and is more alkaline than the full strength of the decinormal "alkaline phosphate" (Na_2HPO_4) in the Sörensen phosphatic mixtures for determination of hydrogen-ion concentration. This means, expressed in the usual logarithmic notation, a concentration of less than P_H, $10^{-9 \cdot 1}$.

This increase of alkalinity is about equal to that shown in 1905 by Moore, Roaf, and Whitley* to promote disordered cell-division, and, later, death in the dividing cells of fertilised echinus eggs. Now, when the sunshine is strong in Spring and Summer, in every pool upon the seashore above half-tide level, when isolated from the sea, such active photo-synthesis must proceed with development of alkalinity, and such changes must have a marked effect upon any animal forms of life in the pool. Such environmental changes, with their stimulating action upon cell-division may play a part in originating deviations and producing variations. In Winter such influences would be slight, but as the Spring days lengthen and the altitude of the sun increases their power augments and reaches a maximum just as reproductive processes and rapid cell-division are at their height. In the experiments recorded below, in order to prevent death from alkalinity, a supply of carbon dioxide was given by blowing air from the lungs through the sea-water in which those algæ were immersed, which were exposed to full day or sunlight whenever the titrations showed an approach to the lethal limit.

The main series of experiments was carried out during the period of March 28th till April 5th, 1919, in two sets of twelve each of the Kilner fruit-preserving jars, each of about 800 c.c. capacity and provided with air-tight screw-down lids, as described in a previous paper.† Afterwards, for purposes of analysis, the two sets at the end were combined and preserved together. As there were two similarly treated jars in each set, the Kjeldahl determinations for nitrogen came to be made upon the contents of four jars; thus a good average was obtained.

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* Moore, Roaf, and Whitley, 'Roy. Soc. Proc.,' B, vol. 77, p. 102 (1905).

† Moore and Webster, *loc. cit.*

The green sea-weed utilised was *Enteromorpha compressus*, which grows abundantly in the open wooden runnels supplying the freshly-pumped-up sea water to the ponds of the Fish Hatchery. This weed is well suited to the purpose because it is easily divided and can be accurately weighed out in definite amount. A quantity of the Enteromorpha was gathered and relieved of excess of sea water by pressing gently between folds of filter paper. It was then weighed out into 0·5-grm. quantities, and one of these quantities was placed in 200 c.c. of fresh sea water in each Kilner jar. Nothing save the weed and water was added to any jar. The first dozen jars were started on the morning of March 28th, and the second set on the afternoon of the same day; they were pickled together in absolute alcohol as preservative on the morning of April 5th, and also the waters in which they had grown were taken away for analysis, with the results given below.

The two sets were treated identically as to exposures, and the first set may be taken as an example for both:—

Nos. 1 and 2.—Kept with lids tightly screwed on in such daylight and sunlight as were available outdoors.

Nos. 3 and 4.—Ditto, but instead of tightly screwed-on lids, these were covered above only with a double layer of muslin, so that there was free access of atmosphere.

Nos. 5 and 6.—Exposed to diffuse light only indoors on shelf in laboratory, with lids tightly screwed on.

Nos. 7 and 8.—The same as to light, but open, with muslin covers only.

Nos. 9 and 10.—Kept in complete darkness in cupboard with lids tightly screwed on.

Nos. 11 and 12.—Also kept in darkness in cupboard with muslin covers only.

At intervals, as noted in the subjoined Table, a quantity of 100 c.c. of sea water was taken from one jar of each type of exposure, four drops of 0·5 per cent. of phenolphthalëin were added, and the water was titrated to neutrality with the results shown. Then 100 c.c. of fresh sea water were added to restore the former volume, and the jar restored to *statu quo* as to exposure. The samples of weed in each set of four jars with identical exposures did not during the experiment ever receive more than 1000 c.c. of sea water in all. Samples of fresh sea water, and of that from the jars open in sunlight, were analysed for amino-nitrogen by Kjeldahl destruction and

Nesslerising at the termination of the experiment, and the amount in each case was found to be 1·3 mgrm. per litre. Also, in each case, analysis for nitrite and nitrate gave in both cases 1 in 10 million to 1 in 15 million expressed as nitrogen. It follows that the nitrogen converted by the growth in light of the algæ could not be obtained from combined nitrogen as ammonia, nitrite or nitrate. The only other available source is dissolved nitrogen in the sea water derived from the air.*

A glance at the fifth column of the Table given below shows that the four jars, closed air-tight but kept in sunshine, fixed nitrogen almost as rapidly as the open jars in sunshine. This furnishes proof that the source of the nitrogen fixed is not the nitrogen peroxide, or so-called ozone, of the air, but the elemental nitrogen. This does not, of course, exclude nitrogen peroxide as a nutrient and a stimulant; experiments in the preceding communication by Moore and Webster on fresh water algæ have shown that dilute nitrogen peroxide can so functionate. But both series of experiments agree in proving that, given an ample supply of carbon—either as carbon dioxide or as bicarbonate, and the presence of light-energy, then elemental nitrogen from the air in solution in the nutrient medium can be fixed and built up into organic compounds. At the conclusion of the series of experiments, the four samples of weed in each set of four bottles were separated from the sea water, united, pressed between filter papers, and the moist weight taken. As at the outset, 0·5 grm. was weighed out into each jar; the initial weight in each set was 2 grm.

After weighing, each set of weed was placed in a wide-mouthed glass-stoppered bottle, and preserved in a quantity of about 80 c.c. of absolute alcohol.

When the analyses were started, the preliminary step in each case was to evaporate off the alcohol in a weighed capsule, add the preserved weed, and dry to constant weight; these dried weights are recorded in Column 4 of the Table. Then each dried weight was analysed for nitrogen by the usual Kjeldahl method, and the results are given in Column 5.

Commentary on Table I.—The figures given in Column 2 show the alkalinity developed by the photo-synthesis. Notice how much greater it is in full light than in diffuse light, and that in complete darkness it becomes negative because the carbon dioxide discharged in oxidative processes in darkness renders the sea water acid to phenolpthaleïn. The normal sea water at this period possessed an alkalinity to this indicator represented by about 2·5 c.c. per 100 c.c. of sea water. The alkalinity tends to rise higher in the "shut" jars in sunshine, because in the "open" jars in sunshine the atmospheric carbon dioxide can enter through the muslin and partially re-neutralise the normal carbonates of magnesium and calcium formed.

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* See note at end of paper.

Table I.—Fixation of Nitrogen by Marine Algæ. Experiment of March 28–April 5, 1919 (seven days' interval).

1. Nature of exposure.	2. Titration in c.c.'s of N/100 acid in 100 c.c. of sea water required to neutralise to phenolphthaleïn.		3. Wet weight at end. Initial weight in each case, 2 grm. in 800 c.c. sea water.	4. Dry weight at end in grammes.	5. Total nitrogen by Kjeldahl. In milligrams.
	April 2.	April 5.			
1. Sunlight, in *shut* jars, outdoors	13·3	9·7	2·57	0·476	11·3
2. Sunlight, in *open* jars, outdoors	6·7	9·7	2·38	0·457	12·4
3. Diffuse light, no direct sun, on shelf indoors. Shut jar	0·4	3·4	1·71	0·284	8·7
4. Same exposure as No. 3, but jars open	0·0	+1·7	1·47	0·285	8·0
5. Darkness, in cupboard. Shut jars	−1·7	−1·4	1·45	0·259	9·4
6. Same as 5, but jar open.........	−1·2	−0·3	1·50	0·275	9·5

It is of some interest to make a rough calculation of the quantities of fixed carbohydrate, protein or fat that these amounts of fixed carbon dioxide, as estimated from the titrated increases of alkalinity, would account for during the period of experiment. Taking the mean of the two amounts of alkalinity upon the two days observed, viz., 13·3 and 9·7, one obtains 11·5. Deducting 2·5 c.c. for the level of normal sea water at this period of the year the result is 9·0 c.c. of centi-normal alkali resulting from photo-synthesis per 100 c.c. This change occurred in 800 c.c. in each set for a period of seven days. This works out at 500 c.c. of centi-normal alkali during the period. Now 1000 c.c. of normal alkali corresponds to 12 grms. of carbon fixed, and on this basis the carbon fixed is 60 mgrm. This would correspond, on the basis of 45 per cent. of carbon in carbohydrates, 60 per cent. in proteins and 75 per cent. in fats, to a fixation of 130 mgrm. of carbohydrate, 100 mgrm. of protein or 80 mgrm. of fats.

Turning now to the increased moist weight during the experiment from 2·00 grm. to 2·57 grm. and taking the dry weight at the end of 0·476 grm., the increase in dried weight works out at 106 mgrm.

There is thus a close correspondence between the increase in weight and that which would be expected, viz.: increase in dried weight found 106 mgrm.;

expected increase in dried weight if all were converted into carbohydrate, 130 mgrm.; expected increase if all protein, 100 mgrm.; expected increase if all fat, 80 mgrm.

If next the increase in the nitrogen be considered, this amounts to about 3 mgrm. which corresponds to about 20 mgrm. of protein, the remaining 80 to 90 mgrm. fixed would therefore represent the carbohydrates and fats.

Addendum on Nitrogen-fixing Bacteria.

Our attention has been drawn to the fact that three different observers* have demonstrated that the surface of the ordinary large sea weeds is regularly colonised by nitrogen-fixing bacteria, and it has been stated that these bacteria have been identified with the nitrogen-fixing bacteria known in soils.

Reinke states that nitrogen bacteria were found without exception on all the marine algæ of Heligoland sent to the Botanical Institute at Kiel for examination. The thoroughly washed alga was placed in a suitable nutritive medium and there followed a heavy development of Azotobacter and corresponding fixing of nitrogen from the air.

In Keutner's experiments small pieces of various algæ were introduced into a culture medium and in about ten days the fluid became turbid and a scum was formed on the surface of the water and on the pieces of sea weed. The bacteria could be identified in the slime on the algæ. Experiments made on fresh-water plants gave the same results. This observer definitely states that the culture flasks were kept either in a closed chest or cupboard or in a thermostat—that is to say, in the dark.

Keding's work is a continuation and development of the foregoing, and the statement that the algæ-colonising bacteria are the same as those known in soils can probably be traced to his finding that the Azotobacter of the Baltic and that of the land "sich wenigstens in den in Betracht kommenden physiologischen Eigenschaften identisch verhalten." This at least is the only evidence that we are able to find for such a conclusion.

With the above results before him, Hermann Fischer† is inclined to attribute to the action of bacteria, in the majority of instances, such cases of fixation of nitrogen by green plants as have hitherto been recorded, and holds that in every case where a claim is made to have observed such fixation a need exists for proof that the technique was perfect.

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* Reinke, 'Ber. deut. bot. Gesell.,' vol. 22, p. 95 (1904); Keutner, 'Wissen. Meeresuntersuch., Kiel,' vol. 8, p. 27 (1905); Keding, *ibid.*, vol. 9, p. 275 (1906).

† "Das Problem der Stickstoffbindung bei Pflanzen," 'Ber. deut. bot. Gesell.,' vol. 35, pp. 423–454 (1917).

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It is to be regretted that we had not the results above quoted before us when starting our series of experiments, and that in consequence no special precautions were taken to exclude nitrogen-fixing bacteria from our jars. Yet in our opinion it may be reasonably assumed that their presence did not seriously affect the results obtained, for the following reasons:—

(1) What we consider to be definitely proven as a result of these and former experiments above referred to is the utilisation of solar energy for the purpose of nitrogen fixation. Column 5 in the Table shows a marked difference in the amount of nitrogen, fixed according as the sea-weeds were exposed to sunlight, or kept in weak light, or in the dark. It appears to us to be a point of secondary importance whether that energy is so utilised by the alga itself or by an associated bacterium. If the effects observed are to be ascribed to the latter, it would be a novel experience to find a chlorophyll-less organism so functioning in relationship to light, and the proof that it could do so would be of unsurpassed interest in relation to the problem of the origin of life in a chlorophyll-less world.

(2) It is to be noted that, in order to get nitrogen fixed in appreciable quantities, previous observers have found it necessary to grow their bacteria in appropriate culture media containing small quantities of glucose, mannitol, or other organic compounds, as well as the inorganic phosphates, etc. These media in due course became turbid, and bacterial scums were formed. Our experiments were carried out in pure sea water only, and, during the eight days that they lasted, there was no sign whatever of bacterial growth. The water remained perfectly clear throughout, and there was no indication of a scum on water or weed, either in the jars exposed to sunlight or those kept in the dark. From this, one may legitimately conclude that the nitrogen-fixing bacteria, if present, had not multiplied to any great extent nor sufficiently to account for the results obtained.

Summary.

1. Marine algæ, like fresh-water algæ, can fix elemental nitrogen from water and thus indirectly from air, in presence of sunlight, but not in darkness.

2. The store of bicarbonates of calcium and magnesium present in sea water furnishes an abundant source of carbon dioxide utilisable for carbon fixation, and as fixation proceeds the sea water becomes more alkaline. The limit of alkalinity is that at which all bicarbonates have become converted into carbonates, and at this point the potential of hydrogen-ion concentration has fallen below the value $P_H = 10^{-9 \cdot 1}$.

3. In the strong sunshine of Spring and Summer this degree of alkalinity

is sufficient to favour increased rapidity of cell-division and induce abnormal and varying forms.

4. Marine algæ grown in a limited volume of water and a limited supply of air in sunlight and full daylight fix both carbon and nitrogen rapidly into organic compounds. The amount of nitrogen fixed exceeds many times the total nitrogen originally present as ammonia, nitrite, or nitrate in the water. Moreover, the small initial amounts of nitrogen present in these forms are not decreased. It follows that the only available source is the free nitrogen of the atmosphere.

Studies on Synapsis. III.—*The Nuclear Organisation of the Germ Cells in* Libellula depressa.

By Lancelot Hogben.

(Communicated by Prof. E. W. MacBride, F.R.S. Received October 15, 1920.)

[Plates 4–7.]

An impressive array of facts in support of the chromosome hypothesis has been elucidated by the researches of cytologists during the past two decades; nevertheless the paucity of direct objective evidence for the integral continuity of the chromosomes through all stages of nuclear history still presents a barrier to unqualified acceptance of its validity. Such knowledge is not only essential to a convincing presentment of the chromosome hypothesis: it has furthermore a very intimate bearing upon the interpretation of synapsis.

The earlier workers, following Flemming, interpreted the prophase and telophase organisation of the nucleus as a continuous spireme: this notion rapidly hardened into a dogma, which not only handicapped further inquiry into the problem of the constitution of the resting nucleus, but proved to be a source of much confusion respecting the significance of the events in the meiotic phase. How far theory even succeeded in overriding fact may be illustrated sufficiently by a quotation from a paper that has exerted a powerful influence on cytological theory.* In reference to the spermatogonial prophases of Periplaneta, the authors state correctly, "the method of chromosome formation here depicted presents nothing exactly comparable to the long spireme thread which is figured in so many existing accounts of the

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* 'The Meiotic Phase in Animals and Plants.'

premeiotic divisions. . ." After this unequivocal statement the interpretation given to account for the reduction of the chromosomes is that "the spireme threadwork tends to separate out into half as many lengths" as the number of premeiotic chromosomes. Now, it may safely be said that in general (as well as in the case of Periplaneta) recent research has fully justified Bolles Lee in his contention that "ni à la télophase, ni aux prophases, ni à aucun moment de l'existence de ces noyaux, il y aît formation d'un spirème continu." Yet even recent workers, like Arnold, 1909 (1), Nakahara, 1919 and Harman, 1920 (2), in their inability to account for telosynaptic union by other means, fall back upon the conception of a spireme segmenting into the haploid number of threads as a means of effecting reduction.

The importance of an understanding of the various phases characteristic of nuclear division was emphasised in the last study in reference to comparisons made between the meiotic processes in Osmunda and Periplaneta. Attention was there directed chiefly to the question of whether the telophasic chromosomes divided in anticipation of the succeeding mitosis. A preliminary examination of spermatogenesis in Libellula in May, 1920, indicated that the material was of a very favourable kind for a more searching study of nuclear organisation in the premeiotic phase; and the present communication contains the results of observations made on material collected at that time and subsequently. The nymphs were identified, by the courtesy of Mr. H. Campion, of the British Museum, as *L. depressa.* Particulars of the materials and methods employed will be given with the observations recorded.

Previous Work on Odonata.

Since the Odonata, as a group, have attracted very little attention hitherto from cytologists, a brief summary of previous work may first be given. The only observations recorded with respect to the chromosome cycle are those of McGill, Lefevre, and Smith.

McGill, 1907 (3) has published an account of the oogenesis of two genera, Anax and Plathemis; the more salient particulars are: (*a*) the presence of yolk nuclei of the Lumbricus type in the young oocyte; (*b*) the condensation of the "synaptic spireme" around the plasmosome to form a double nucleolus; (*c*) the emission of fluid particles from the double nucleolus during the growth period of the egg. The author speculates that these latter are "reprecipitated" after "undergoing chemical change" to form the chromatin of the mature oocyte. The alleged condensation of the "synaptic spireme" around the plasmosome is homologised with synizesis in the male sex cells; and the views of Retzius, Leydig, Strassburger, Guignard, and Mertens, who regarded the nucleolus as a temporary form of chromatin storage, are believed to be reinforced by the study of the phenomena cited. Despite the unusual character of the process described and the interpretation inferred, no other investigator has repeated this work.

In 1908 Lefevre and McGill (4) repeated the earlier work of the latter on the spermatogenesis of *Anax junius.* In their amended form the chief points of interest recorded are as follows: (*a*) the diploid complex of the male germ cells in *A. junius* consists of twenty-seven chromosomes (including twelve pairs of rod-like, one accessory, and a pair of micro-chromosomes); (*b*) the chromosomes of the spermatogonial telophase form a continuous spireme; (*c*) the long axis of the tetrad is equivalent to the line of cleavage of the diplotene filament; (*d*) the X-element is segregated in the homotype mitosis.

More recently, Smith, 1916 (5) has made a careful study of the male sex cells in *Sympetrum semicinctum* and *Libellula basalis.* In both species the spermatogonial metaphase displays twenty-five equal chromosomes; the tetrads are stated to be formed like the rings of the Periplaneta type and to divide in the same way; the accessory is said to pass over undivided in the first reduction division of *L. basalis,* but in Sympetrium to behave as in Anax.

The Spermatogonia.

From the examination of gonads taken from spring and autumn nymphs, it appears that *L. depressa* does not complete its life cycle in less than two entire years. Most favourable material for the study of spermatogenesis is obtained from nymphs in the early spring immediately preceding the final moult. The testes then consist of cysts of spermatogonia undergoing mitotic changes and of spermatocytes; a remarkable feature of the species is the very short duration of the resting stage of the nucleus. In very young cysts, composed of only a dozen spermatogonia, the dimensions of the nuclei considerably exceed those of the nuclei of cysts of numerous cells; and, as the cells are in any case small, the former provide most suitable objects for observation. While it would appear that the divisions follow rapidly upon one another, a circumstance which facilitates observation, it is rarely found that the cells of any particular cyst differ to any marked extent in the phase of nuclear history which they display; this fact is again helpful to an intensive study of individual stages. The material employed was fixed in Flemming's reagent (either with or without acetic acid), diluted with two volumes of aq. dest. For staining, thionin, Gentian violet, and Haidenhein's iron-alum hæmatoxylin were used.

The history of the nucleus of the spermatogonial cells may be commenced most conveniently at the metaphase. As more than a hundred individuals were obtained for this purpose, an abundance of preparations, with all the chromosomes well separated in the equatorial plate, were available; in every case there were seen to be twenty-three stout, rod-like and slightly curved elements of approximately the same size. In spite of the fact that Smith gives the number twenty-five for the diploid complex of *L. basalis,* it is without the slightest hesitation that the former figure is stated as the correct count in the case of *L. depressa.* In many preparations (Plate 4, fig. 1) there are seen two (or sometimes one) small granules among the metaphase chromosomes; these occur also in the metaphase figures of follicle cells and oogonia (figs. 26–7); and it was at first an attractive supposition that these may be the vestigial representatives of the additional pair of chromosomes in *L. basalis,* especially in view of the fact that Lefevre and McGill describe a pair of *m*-chromosomes in *Anax junius.* This possibility was fully explored; and it was found that three such bodies were present in many cases in prophase nuclei of spermatogonia, while one, two, or even three were present, separated well apart in the first spermatocyte metaphase (fig. 22). It will be remembered that McGill originally could only find one "*m*-chromosome" in Anax; and the inconstant number of these granules, taken in conjunction with their failure to segregate in the reduction division, makes it very improbable that they can be justifiably interpreted as elements of the diploid complex in *L. depressa* at least.

An alternative proposition, that they constitute a group of Y-elements, is disposed of by comparison with the oogonial nuclei. In the prophase, these granules are first seen at the time when the plasmosome disappears; and there are considerable indications that they represent persistent remnants of the nucleolus passed over from one cell generation to another (fig. 10). One of the most interesting discoveries made with the aid of the new technique is that of Carleton, 1920 (7), who has shown that one or more intranucleolar elements capable of fission in mitosis pass over from one cell generation to another, and constitute the centre around which the new plasmosome is formed in the late telophase. In the light of this discovery, as well as in view of the facts mentioned, the last supposition appears to be the most acceptable.

As the chromosomes pass to the poles of the spindle, they are seen to become more curved; they also give clearer evidence of individuality in size; in the late anaphase (fig. 2) they become more sharply curved, and one or two pairs are seen to become attenuated into looped filaments. There is no evidence of cleavage either in this stage or in that which follows. When the nuclear membrane is defined, the chromosomes are seen to spin out into finely beaded loops, the extremities of which are polarised on the side of the nucleus remote from the centrosome (figs. 3, 4). This polarisation is inevitable if the arc-shaped chromosomes of the anaphase are to retain the same axis of symmetry with respect to the achromatic spindle during the process of attenuation. As the telophase filaments become more elongated, their arrangement becomes increasingly more difficult to follow (figs. 5, 6). The chromatic filaments, however, at all stages remain in contact with the nuclear membrane; whereas the plasmosome, which may appear early in the telophase, while the individual chromosomes are still identifiable as such, is always seen to be in the centre of the nucleus. There is thus no justification for regarding the plasmosome as in any way connected with chromatin organisation of the nucleus.

If the history of the prophase chromosomes is traced chronologically backwards, it is seen that their earliest precursors that can be definitely identified are in the form of attenuated, finely-beaded and convoluted filaments. As these become abbreviated, they show at first a definite orientation with respect to the plasmosome (figs. 7–10). At no stage either in prophase or in telophase is there any justification for regarding the individual filaments as united in a continuous spireme. The history of the chromosomes in the prophase recapitulates in obverse sequence the behaviour of their representatives in the preceding telophase. In this essential, the facts are in agreement with the conclusions drawn by Digby as regards Osmunda, 1919. But there is no evidence of cleavage for the ensuing metaphase, until the prophase chromosomes begin to assume the arc-shaped contour characteristic of the anaphase (figs. 9, 10). There is only one stage, of comparatively brief duration, in the germ nuclei of Libellula, when the individuality of the chromosomes is obscured; this is the so-called reticulate stage, or, as Bolles Lee has called it, the *spirophase;* and it will be noticed that there is no reason to infer that the individuality of the chromosomes is interrupted at this point. On the contrary, the phenomena described would more naturally lead to the conclusion that *the spirophase is in reality merely that point in the nuclear cycle, when the chromatin filaments become so elongated, and the chromomeres so widely separated that current methods of observation can no longer detect the direction of individual strands.*

The only recent account of the behaviour of the chromatin organisation of the nucleus during interkinesis on the Hexapoda is given by Schaffer, 1919 (8). In Lachnosterna (Coleoptera), " the chromosomes spin out into fine chromatic threads, and, as the nucleus grows, the threads become more and more complex . . . this resting nucleus is of relatively short duration, for soon the chromatin begins to condense into heavier threads." One very important resemblance between the events of the nuclear cycle in Libellula and Lachnosterna will be described later. While in general the few workers who have studied the interkinetic nucleus of late years agree that there is no loss of individuality in the "spirophase," the precise details of the process do not appear to be universally alike. In view of the importance attributed in Morgan's suggestive speculations to the linear alignment of the chromosome segments, it is tempting to seek for an interpretation of the spirophase in other forms along lines similar to those indicated above. But at present there is no justification for doing so.

There seem to be at least two distinct types of nuclear organisation in the resting stage. The first may be termed the *unit-reticula-complex;* in this case the telophasic chromosomes become increasingly vacuolated, until the spongy cylinders so formed cannot be distinguished, This at least is certainly the case in certain Orthoptera (*cf.* of Mohr, Buchner, et al.), where the individual reticula of the telophase chromosomes are separately invested in their own membranes; it is also the only possible interpretation of the diffuse stage in the oocyte.* The second type of resting nucleus is that to which the term spirophase was applied by Bolles Lee, 1912 (9), as the result of his very important work on *Paris quadrifolia.* With the exception of Dehorne, 1911 (10), workers of animal cells are agreed that there is no cleavage in the telophase threads; many botanical cytologists like Fraser, 1912 (11), and more recently Digby, adhere to the belief that the cleavage, which becomes manifest at one mitosis, begins in the anaphase or telophase of the preceding mitosis; but others, like Gates, 1912 (12) and Bolles Lee, find no evidence of preparation for the ensuing division until the prophase itself. One thing is evident in any case: the prodigious heterogeneity of structure of the chromosomes in the interkinesis as contrasted with their visible compactness of structure in the metaphase. And this should be borne in mind in reference to certain criticism which has been raised in reference to Morgan's hypothesis by Prof. Bateson in his recent Croonian Lecture.

The Meiotic Phase in the Male Germ Cells.

Lefevre and McGill do not give details respecting the initial processes during the synaptic period† in the primary spermatocytes of Anax. In the case of Sympetrum and *L. basalis,* Smith records the formation of leptotene filaments from irregular blocks of chromotin, as in Oncopeltus and Lygaeus (Wilson), following on a short reticulate phase. In no preparations of

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* Except perhaps in *Hymenoptera parasitica* (Hogben).

† American authors use the term "growth period" more conveniently applicable to the period when the oocyte grows in cytoplasmic bulk and yolk deposition occurs. "Maturation prophase" is better restricted for the formation of the definitive tetrads, especially in view of the telophasic significance of the earlier events. "Meiotic phase" includes both reduction divisions. Hence the use of a special term.

Libellula depressa was it possible to find any such stages. As already mentioned the telophase of the spermatogonial mitoses is characterised by an orientation of the filaments exactly like that which is found in the bouquet stage in Periplaneta; and in fig. 13 of Schaffer's paper (8) precisely the same occurrence is clearly illustrated. All the available evidence points to the conclusion that the *fine polarised leptotene loops of* Libellula depressa *display from the inception of the meiotic phase the arrangement characteristic of the chromatin filaments in the premeiotic telophase* (*cf.* figs. 4 and 11). As far as could be ascertained the leptotene filaments are from the first present as loops polarised at both extremities (fig. 11). It is very difficult to count the loops with accuracy; but their number is clearly more numerous than in the pachytene stage. In the contraction figure, they are drawn away from the nuclear membrane and become considerably abbreviated (fig. 13). Owing to their crowded disposition, it is not possible to observe with certainty actual pairing; but it is certain that throughout this stage and in that which follows, when it is possible to count with accuracy the haploid number of loops, they retain the original condition of polarisation at both extremities (figs. 13, 14).

Now if the polarisation of the leptotene loops is consequent upon the character of the last premeiotic telophase, there is no possibility in the case of Libellula that numerical reduction of the loops could be effected by means of an end-to-end union without an entire reorganisation of the chromatin organisation of the nucleus. In the absence of evidence for the intercalation of such a process, those who advocate telosynapsis for Hexapoda fall back on the conception of a continuous spireme; envisage reduction by the segmentation of the spireme into the haploid number of lengths: and declare themselves unable to find a previous (leptotene) stage in which the number of loops is more numerous. This contention is based on a fundamental misconception as to the nature of the telophase: when this is rightly appreciated, it follows that conjugation can only take place at one point in the sequence of meiotic phenomena—the bouquet contraction, and can only be effected by one means—the lateral approximation of the loops or filaments.

With respect to the diplotene stage (figs. 14, 15) in which the reduced filaments become extended and display longitudinal cleavage, it is only necessary to state that the nucleus as a whole undergoes considerable increase in bulk during this period (figs. 15, 16). Eventually the haploid number of longitudinally split filaments are detached (fig. 16); and their transformation into the definitive tetrads coincides with a progressive shrinking in size of the nucleus (figs. 17, 18).

The fate and constitution of the tetrads in the Odonata has provoked controversy. That they are formed by the opening out of the line of cleavage of the diplotene filaments is clear (fig. 17). But since some of the tetrads at the time when the nuclear membrane disappears, already exhibit the form of practically symmetrical crosses (fig. 18), there is difficulty in referring the plane of division to the axis of the filament from which they are derived. If the long axis of the tetrad when arranged on the achromatic spindle corresponds to the line of cleavage in the diplotene stage, the first division is reductive in the modern sense, and must separate univalents united end to end: otherwise it is reductive only in the sense in which Weismann interpreted meiotis, dividing each univalent transversely. In either case such a disposition of the univalents in the metaphase of the heterotype mitosis would be incompatible with the theory of parasynapsis as now understood. This is the arrangement described by Lefevre and McGill for Anax; but it is clear from their figures that the sequence of the stages on which they base their contention is an inversion of the correct order. Smith adopts the alternative view in the case of Sympetrum and *L. basalis*; that is, the tetrad is so placed in metaphase that the transverse division of the tetrad involves the separation of the originally longitudinal halves of the diplotene segments. Though it is impossible to give objective demonstration of the correctness of this description for all the bivalents of *Libellula depressa*, at least two of them (*cf.* figs. 19 and 20) are symmetrical about one axis only, and show subterminal instead of median spindle fibre attachment. These are placed in the metaphase with their single axis of symmetry in the equatorial plane, and there is therefore no doubt whatever in regard to these that the heterotype division separates longitudinal halves of the diplotene bivalents. The similarity of these tetrads to the remainder in all other respects permits the inference that the latter behave in the same way.

In metaphase each bivalent is eventually draws out, as is Periplaneta, into an elliptical figure; and as each division completes itself the two arms of the dyads are drawn into closer contact. The homotype mitosis follows quickly on the telophase, and the chromosomes of the second division are actually not more than half the size of those in the first. Consequently it is not an easy task to follow out the prophase of the former, owing to the very small dimensions of the nuclei. A resting phase is not described by Lefevre and McGill in Anax, nor by Smith. But as far as *L. depressa* is concerned, there is such a stage, though of very short duration. The telophase chromosomes of the heterotype mitosis seem to spin out into loops as in the spermatogonia. The prophase chromosomes show cleavage lengthwise. Thus the homotype mitosis is equational, and cannot be referred to the transverse constriction of the dyad. This is exactly as described by Morse and the author in Periplaneta.

Throughout the foregoing account no mention has been made of the behaviour of the unpaired chomosome of the spermatogonia. As will become evident later, this chromosome represents the X-element or accessory of other forms. Throughout the entire synaptic period it can be identified as a club-shaped body (figs. 11, 15), becoming abbreviated in the metaphase I. In every case twelve chromosomes could be counted in the metaphase of the first heterotype division: usually they are arranged so that a central group of three is surrounded by a group of nine, including the accessory (fig. 22). In lateral views the X-chromosome can never be seen to pass to one pole undivided, but appears to undergo division rather later than the bivalents (fig. 21). Metaphase counts, however, of the homotype division display in some cases twelve, in other cases eleven chromosomes (figs. 23, 24). Their arrangement is generally the same as in heterotype metaphase, *i.e.*, an outer ring of nine (or eight) and an inner group of three. Lateral views indicate that the X-element passes to one pole undivided in the second reduction division of *Libellula depressa*, as in Sympetrum and Anax (fig. 25). Smith thinks, however, that this is not the case in *L. basalis* : it would nevertheless be remarkable if such a difference were confirmed in two species of the same genus.

It follows that in *Libellula depressa* the second division is reductional for the accessory chromosome. It has been stated by several writers of the parasynaptic school that where both divisions are longitudinal with respect to the diplotene filaments, it is impossible to identify one or the other as that in which the segregation of the autosomes is effected. But unless the pachytene filaments rotate about their long axes in the contraction phase, the line of cleavage in the diplotene stage must be in the same plane as that in which the conjugating elements approximate. It is, therefore, most reasonable in the absence of evidence to justify such a supposition to assume that the mitosis which separates the longitudinal halves of the diplotene segments is that in which the segregation of homologous chromosomes takes place. In general the pre-reductional view is the more satisfactory: but in the case of the Ascarid tetrad of which each joint corresponds to a longitudinal fissure of the bivalent filament, it is possible that either pre-reduction or post-reduction occurs. Possibly in the latter case the segregation of some of the bivalents occurs in one division and some in the alternate mitosis. The genetic ratios inferred would be the same.

Synapsis in the Oocyte.

Attention was originally attracted to a study of the gametogenesis of Libellula, on account of the remarkable phenomena described in connection with synapsis in the oocytes of two other genera of Odonata by McGill. Examination of the living ovaries showed that the nucleolus of the oocyte is relatively of enormous size; and it exhibits usually a double structure, having a central large vacuolar area; in addition, there is usually an extranucleolar body lying free in the nucleus. With a view to obtaining further data respecting the behaviour of the plasmosome in oogenesis, and with a desire to test the validity of McGill's interpretation of the similar double nucleolus of Anax and Plathemis,* the present research was begun; in the course of the work other points of interest emerged, as described above.

The point of signal interest in McGill's paper is the contention that the double nucleolus of the growing oocyte is formed by the condensation of the synaptic spireme (*i.e.*, bouquet) round the plasmosome. This process is homologised with synizesis in the male germ cells. The definitive chromatin organisation of the later egg is supposed by McGill to be formed by precipitation of substance secreted as globules by the nucleolus and dissolved in the karyolymph. The whole series of phenomena, it is maintained, reinforces the now generally discarded belief of Retzius, Leydig, Strassburger, and others, who regarded the plasmosome as a means of storing chromatin.

This is clearly at variance with (1) the general hypothesis of chromosome individuality, and (2) most accredited data bearing on the meiotic phenomena of oogenesis. It is usual to find in oogenesis, as in the case of Periplaneta, or Gryllus, that the diplotone filaments or pro-tetrads tend to become increasingly granular as the oocyte enters upon the phase of active growth in cytoplasmic bulk, so that typically the whole surface of the oocyte nucleus appears to become covered with uniformly distributed microsomes (chromatin granules) during the major part of the history of the egg. Eventually, the onset of the reverse process occurs, when the polar spindle is formed, *i.e.*, about the time the egg is laid or fertilisation occurs. In insects, with the exception pre-eminently of *Hymenoptera parasitica* (Hegner, Gatenby, Hogben), maturation does not begin in the ovarian egg. In a few cases, as in certain Amphibia, there is no "diffuse stage" at all (Jordan, King); the bivalents persist without such change throughout the entire growth of the oocyte. In yet other cases the bivalents become very diffuse, but are still individually recognisable, as in Pristiurus.

From these circumstances, and the fact that the chromatin units always reappear in the prophase, in the same manner and the same form as that in which they pass into the reticulate condition, it seems legitimate to infer, in the absence of evidence to the contrary, that there is no actual loss of individuality in the chromatin organisation of the oocyte. Evidence to the contrary has indeed been submitted and criticised in a previous contribution to this series, and further data bearing on this question will be considered later. The meiotic phenomena of *Libellula depressa* will be described first, and the question of the "chromidia" discussed in due course.

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* The genus Plathemis has now been merged in Libellula.

The ovaries in Libellula, as in Anax and Plathemis, are a pair of spindle-shaped capsules, enclosing numerous minute ovarioles. They are thus of the type found in Gryllus, consequently, they are not so amenable to very delicate fixation as are the freely-exposed egg tubes of Periplaneta. McGill's material was fixed whole—after dissection in fluid—in Flemming's reagent (strong formula). Comparison with preparations of Periplaneta showed that this technique is totally inadequate when attempted with the Libellula material. Improvement was obtained by adopting three precautions: first, the ovaries were removed without immersion in fluid and plunged directly in the reagent; secondly, they were teased out quickly and thoroughly, so that each ovariole was separately and instantaneously fixed; lastly, the fixing fluid (Flemming's) was diluted to a third the usual strength. When these precautions are observed, the results are in every way more satisfactory. *Mutatis mutandis* this applies to material preserved in chromosmic without acetic acid, and by the Mann-Kopsch method (*vide infra*) for the examination of mitochondria.

The ovarioles of Libellula, Anax, and Plathemis are of the simple type found in Gryllus and Periplaneta: the greater proportion consists of oocytes, progressively arranged with respect to size in linear series. The remainder consists of young oocytes at the inception of growth, lined side by side, a mass of oogonia and oocytes in synaptic stages, and a terminal filament of smaller cells. There are no nurse cells: the follicle cells arise from the primary oogonia and divide mitotically. A few authors have described amitotic division in follicle cells of Hexapoda, but in the majority of cases, probably all cases in which the follicle cells are germinal in origin, they divide in the usual way.

To obtain mitotic stages of oogonia and oocytes in synapsis, the second-year nymphs provide most suitable objects for study; for observations on yolk formation, the third-year nymphs, immediately before emerging, must be obtained. Oogonial mitoses are rare, and the chromosomes of the follicle cells are small and crowded. Hence it follows that the female germ cells do not provide suitable material for investigating the phenomena of nuclear division. A limited number of very clear oogonial anaphase, telophase, and prophase figures were obtained in the preparations studied, and in the main the series of phenomena seems to be essentially identical with what occurs in the spermatogonia. That is, in anaphase the chromosomes become more bent as they pass undivided to the poles of the spindle; in telophase they spin out into loops that are apparently polarised; in prophase the individual chromosomes are first recognised as attenuated filaments, progressively shortening into stout rod-like elements and undergoing cleavage simultaneously. The same is true of the follicular nucleus: in prophase it is usually seen that the separate chromosomes display appreciable differences of size *inter se.*

A few equatorial sections of nuclei in metaphase, both from follicle cells and oogonia, showed the chromosomes sufficiently widely separated to permit an accurate count of the diploid complex. There are in such cases twenty-four univalents: the unpaired element of the male germ cells is accompanied by a homologous partner (fig. 17). Minute deeply staining granules are seen among the chromosomes; but it does not appear that their number is constant, and there are suggestions that they are derived from the nucleolus in the prophase (fig. 26). The evidence in *Libellula depressa* is against the existence of a pair of *m*-chromosomes like those described by Lefevre and McGill in Anax.

At the inception of the meiotic phase the chromatin organisation of the nucleus consists of finely beaded, delicate, polarised loops in contact with nuclear membrane along their entire length. This condition is of comparatively long duration in the case of the oocyte, since it is found with greater frequency than other stages. Even in good sections it is difficult to count the number of loops with great accuracy; but by the study of sufficiently thin sections (3 μ) it is certain, that approximately, the full diploid number of filaments is present. Thus it corresponds to the leptotene stage of Von Winiwarter. It is illustrated in fig. 28. A plasmosome is always seen—usually towards the polar side of the nucleus where the leptotene filaments converge. There is no striking difference among the latter which would lead to the identification of the accessories in the early meiotic stages. As in so many other cases where the male X-element is conspicuously recognisable in synapsis, the accessories of the female germ cells behave in all respects like the autosomes. The only qualification which this statement merits is that in the metaphase of follicular mitoses two chromosomes are distinctly larger than their fellows.

The bouquet figure now passes through the stages of contraction seen in Periplaneta: the leptotene loops are withdrawn from the nuclear membrane, become shorter and thicker, and are in consequence more closely approximated side by side. There is one noticeable difference between the two cases; in Libellula, as in Tomopteris, the process is not synchronous for all the elements concerned; hence, while it is difficult to state dogmatically that there is a parallel conjugation in pairs of all the loops, it is possible to affirm without any shadow of doubt that such is the case with those which lag behind the rest (fig. 29).

In such cases it can be seen, as might be anticipated from the orientation of the loops, that approximation begins at the polarised extremities of the filaments extending distally till there is only a small portion where they still remain apart. The thickening of the filaments and their reduction numerically, as will be seen later, is effected in this process, and a consideration of the disposition of the loops makes it evident that reduction could only be brought about by parasyndesis. Though the loops, now bivalent, contract considerably towards the polar side of the nucleus with plasmosome to the side of or in the centre of the bouquet figure (fig. 30), in really good preparations there is nothing strictly comparable to the compact synizesis of some authors. A comparison between what occurs in Libellula and McGill's figures of Anax and Plathemis will suffice to make clear that what is described as the condensation of the synaptic spireme round the nucleolus in these two genera corresponds to maximal contraction in Libellula (fig. 30). Indeed, precisely similar appearances to those described by McGill are seen in preparations of Libellula made originally by the technical methods employed by that investigator.

The chief hiatus in McGill's account of the meiotic phase of the oocyte in Anax and Plathemis concerns the subsequent events. And there can now be little doubt that this is due to the short duration of the post-synaptic stage which intervene before the diffuse condition of the oocyte nucleus. It is a curious fact that, whereas in probably the majority of animals, the diplotene stage is that of longest and leptotene of shortest duration in the series of meiotic phenomena, the immediately post-synaptic stages in the oocytes of Libellula are extremely rare compared with the earlier ones. Thus a liberal supply of nymphs must be available if the complete history is required. And here opportunity arises to reiterate the paramount necessity of examining very large quantities of material for elucidating the whole sequence of events in the nuclear history of the germ cells: probably there is no more common occasion for error that has arisen in connection with the work which has been published in relation to the controversies which centre round the interpretation of synapsis than the extreme rapidity with which in certain cases some of the most critical stages are passed. For this reason negative evidence such as the failure of Nakahara and Arnold to find typical leptotene stages cannot carry the conviction which its exponents appear to anticipate.

After remaining for some time in maximal contraction, the pachytene loops become sufficiently loosened to permit exact numerical estimation (fig. 31) in sections at right angles to the axis of the bouquet: it is then seen that only the haploid number are now present. Numerical reduction is thus effected with certainty in the contraction process. As the pachytene loops extend into contact with the nuclear membrane (fig. 32), the longitudinal cleavage of the diplotene stage becomes evident. There follows the dissolution of the bouquet; and finally the abbreviation of the diplotene loops into segments of which the longitudinal halves become drawn apart except at their extremities (fig. 33). These latter condense to form tetrad-like bodies, which soon become increasingly granular in appearance, so that individually they are no longer recognisable, the surface of the nucleus in good preparations being apparently evenly covered with chromatin granules. Thus the diffuse stage supervenes in Libellula as in Periplaneta; and throughout the entire series of events the plasmosome remains altogether independent of the chromatinic organisation of the nucleus (fig. 35). Thus it is necessary to seek for some alternative interpretation of the double nucleolus of *Libellula depressa* to that offered by McGill for Anax and Plathemis; and when the subsequent history of the oocyte nucleus in Libellula has been stated, there will be found little cause to doubt that the same procedure is in reality to be found in the other two genera.

Before passing on to an examination of the genesis and significance of the double nucleolus, it is interesting to compare the chromosome cycle of the Odonata with that of other Hexapoda. The early differentiation of the tetrads and the retarded segregation of the X-element in the male germ cells is similar to what has been described by Wilson for several genera of the Hemiptera. On the other hand, the definite polarisation in the synaptic stage conforms to the Orthopteran type. In Libellula, as in Periplaneta and Gryllus, the initial meiotic stages are of longer duration in the female germ cells than in the spermatocytes; also, owing to the larger size of the nuclei, the former are more favourable for investigation; it is therefore important, in the interpretation of synapsis, that work on the gametogenesis of both sexes should go hand in hand. It is true that, as the parasynaptic view has gained in prestige, some authors, *e.g.*, King (13), have pressed the compromise suggested by Gates (12) to the point of advocating both metasyndetic and parasyndetic reduction not only in closely allied species, but in alternate sexes of the same species. This position may satisfy those whose attention has been focussed exclusively on the frequently very intricate task of elucidating the complicated metamorphosis of the spindle bivalents, the conjugant halves of which in the final stage are often united only by one extremity. But, as Agar has rightly insisted, a survey of the entire sequence of changes reveals such striking similarity, more especially in the universally characteristic diplotene stage, that a satisfactory common ground is not easy to find for cytologists of the conflicting schools of opinion by any such facile means. Furthermore, as

Agar points out, in so many cases where earlier accounts (many Orthoptera, Platyhelminthes and Amphibia) appeared to support the telosynaptic interpretation, later examination of the very same animals provided evidence of parasynapsis.

Even accepting provisionally the possibility of pairing of both types in reduction among different animals, a difficulty emerges from the study of the premeiotic prophase and telophase, which to the author's knowledge no animal cytologist, who has of recent years advocated telosynapsis, has attempted to dispel. That many plant cytologists have succeeded in doing so is well known; and it has already been insisted in a previous paper that the similarities of the meiotic phase in animals and plants may have been grossly exaggerated. The possibility of relating the leptotene stage to the previous telophase in animals emphasises the dissimilarity further: that is, if actual synapsis in plants accompanies second contraction. *Since modern research has failed to find any confirmation for Flemming's continuous spireme, the task devolves on those who adhere to terminal as opposed to lateral conjugation in animals to state (in terms consonant with the known behaviour of the chromosomes in the meiotic and premeiotic nucleus) at what precise point this process is supposed to take place.*

The So-called Double Nucleolus.

Though the belief in the individuality of the chromosomes as persistent units of cell structure increasingly finds favour among those who study the problems of the cell, it is idle to overlook the fact that many current observations are clearly antagonistic to it. One such has been dealt with in the previous paragraph; a second, the question of the "chromidia" and so-called secondary nuclei has been touched upon in previous studies (6). On this point the behaviour of the plasmosome in the oogenesis of Libellula (figs. 36-39) is specially instructive; and a brief consideration of it will make clear how McGill was misled in describing the origin of the double nucleolus of Anax.

A reference to Jorgensen's work (14) will amply illustrate the capricious changes in staining capacity of the plasmosome accompanying the growth of the oocyte. Most commonly the nucleus of growing oocytes in preparations of Libellula fixed with Flemming's reagent and stained with Auerbach's method, exhibit a nucleolus consisting of a large medullary portion which is fuchsinophil and a surrounding cortex stained with methyl green. Precisely the same type of nucleolus is described by McGill, who failing to find typical stages succeeding contraction and led astray by the belief that fuchsin and methyl green are an infallible means of differentiating chromatin from other cell constituents, concluded that the double nucleolus is formed by the condensation of the synaptic spireme around the plasmosome during maximal contraction. But complicated methods of differential staining cannot suffice to shed light on cell problems, unless proper attention is paid concurrently to the technique of fixation; and McGill's materials were entirely fixed in fluids containing acetic acid. As a matter of fact, the plasmosome is uniformly fuchsinophil after fixation with Flemming's modified formula (Gatenby), *i.e.*, unless rendered basophil by previous treatment with acetic acid; this is true of all stages in oogenesis, both for Libellula and Periplaneta. There is no need to recapitulate previous comment (6*b*, pp. 315–319) on the value of differential staining; the conclusions built up within the scaffolding of the now discarded binuclearity hypothesis indicate the sources of error that arise from data derived in this way only.

Having examined living material in Libellula, it is possible to state with greater confidence what occurs in the nucleus during the diffuse stage. At the inception of the period of growth, after fixation in Flemming's, Bouin's, or Tellyesnicsky's fluids, the plasmosome, which undergoes at this time rapid increase in size, is seen to have changed its staining reaction. This may merely be a physical consequence of increase in surface (*cf.* Fischer's granules); and does not necessarily denote alteration of chemical constitution. Intranucleolar vacuoles make their appearance and increase in volume *one at a time*, each one successively enlarging, until it occupies the greater part of the plasmosome, while simultaneously becoming oxyphil in its reaction. *Thus the apparent double nature of the plasmosome results not from the absorption of any part of the chromatinic organisation of the nucleus, but from a process of internal differentiation.*

The only difference between the behaviour of the plasmosome in the oocytes of Libellula and Periplaneta in this respect is that the vacuolar bodies within the nucleolus attain their full size singly in the former, whereas several mature at a time in the latter. Their subsequent fate is similar in both genera. Each of these vacuolar bodies, which for ulterior reasons as well as to avoid the confusion between chromatin, mitochondrial, and nucleolar substance suggested by the older term "chromidia" have been designated deutosomes (6), emerge from the plasmosome into the karyolymph. According to McGill in the case of Anax and Plathemis they dissolve in the karyolymph; but in preparations made with the mitochondrial technique of Gatenby and Kopsch they are found to pass out into the cytoplasm to the periphery of the egg, where they eventually break up into smaller spheres which become the first vitellus (fig. 39).

Since the nuclear origin of the mitochondria has been discredited, cytologists have been very hesitant to admit the passage of material from the nucleus to the cytoplasm; partisans of the mitochondrial and chromosome hypotheses have been equally eager to disclaim any such suggestion. That the plasmosome plays an important metabolic *rôle* in oogenesis, and in a number of cases actually secretes fluid or solid particles that pass as such into the cytoplasm is, however, clearly indicated by the recent work of Hempelmann (15) and Buchner (16) on the *Archiannelid Saccocirrus*, Dendy on *Grantia*, and the testimony of numerous writers who have lately examined the secondary nuclei of the Hexapod egg. While some confusion arises by the use of the term "chromatin" (17) for such particles, since structurally the plasmosome and chromatin are separate and no valid chemical tests indicate their identity, it now seems possible to place the whole question of the transmission of nuclear material to the cytoplasm with the genesis of the secondary nuclei upon a basis which is not incompatible with what is generally accepted regarding the behaviour of chromatin and mitochondria in the germ cycle.*

Buchner, in particular, traces the origin of secondary nuclei in the Hymenoptera to the plasmosome; and the author has recently examined preparations of the ovaries of Crœsus, one of the Tenthredinidæ (unpublished), in which this is certainly the case.† Now if the plasmosome is to be added to the list of independent cell organs, as Carleton's work in itself strongly indicates, it may seem at first highly improbable that bodies so closely simulating true nuclei as do the accessory nuclei of the Hymenoptera should not be chromatinic in origin. Though characteristic secondary nuclei are not found in Libellula, the behaviour of the plasmosome in this form, as also in Anax and Plathemis, is here of great interest. It will be remembered that the intranucleolar deutosomes of Periplaneta were said to have the appearance of minute nuclei. In the nucleolus of Libellula, the oxyphil medulla (intranucleolar deutosome) is at first of finely granular structure in fixed preparations. This is indicated in figs. 36, 37, but in illustration its extraordinary resemblance to a nucleus cannot be reproduced in such a way as to do justice to the original. In short, *the oogenesis of Libellula shows that bodies may be formed within the plasmosome itself which have as genuine a resemblance to true nuclei as the secondary "nuclei" of the Hymenopteran egg: this reinforces the evidence for regarding the latter as a product of the plasmosome; and the undoubted emission of nuclear material during oogenesis no longer necessitates*

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* Cytomixis in Œnothera (Gates) merits re-investigation from this standpoint.

† By the courtesy of Dr. Gatenby I have recently seen preparations of Apanteles which show vacuolation of the plasmosome and presence of accessory nuclei in the diplotene stage; this leaves no doubt, in my mind, that the latter are nucleolar rather than chromatinic in origin.

the view that the integral continuity of the chromatinic organisation of the nucleus is interrupted in the diffuse stage.

The Yolk Nucleus of Libellula.

In concluding this record of the history of the ovarian oocyte in *L. depressa* reference is due to a crescentic body found in a juxtanuclear position in Anax; this is stated to undergo fragmentation and dispersal in the cytoplasm of the egg as growth proceeds. McGill calls it the yolk nucleus on the analogy of a similar structure described by Calkins and Foot in the egg of Lumbricus and Allobophora. Such a body is also present in the oocytes of *L. depressa.* After the examination of preparations fixed in Flemming's modified formula (without acetic acid), and by the Mann–Kopsch method,* followed by turpentine to remove osmicated fat, it appeared that the yolk nucleus is in reality the mitosome or chondriome of the oocyte, being the area in which the mitochondria are congregated. This not infrequently resists injurious fixation in young oocytes—perhaps because the protein component still differentiates from the ground cytoplasm after the lipins have been extracted. Gatenby has described the dispersal of the mitochondria in the oogenesis of Apanteles recently; and the facts are essentially similar in Libellula, except that in the latter the mitochondria do not appear to become filamentous. An interesting point noticed was that in some cases the spherical bodies emitted from the plasmosome and lying in the periphery of the egg outside the mitochondrial area were sometimes seen to be invested with a cloud of mitochondrial granules (figs. 38, 39).

The work of Gatenby (17), Hirschler (18) and others has shown quite clearly that both mitochondria and dictyosomes (Golgi rods) may be transformed into deutoplasmic spheres. On the other hand, Gatenby and Nusbaum Hilarowicz (19), who has recently demonstrated a Golgi apparatus in Dytiscus eggs, were not able to trace the origin of the yolk in the Hexapod egg to the cytoplasmic inclusions. Is it therefore necessary to conclude that the mechanism of yolk formation with respect to the cell organs is totally different in different animals? The observation recorded above seems to offer an alternative. Jorgensen's researches, though unfortunately largely vitiated by inadequate technique, do at least show that yolk formation throughout the animal kingdom coincides with a period of intense metabolic activity on the part of the plasmosome; it is equally certain that the process of yolk formation in general corresponds with a period of rapid increase in number of the extra-nuclear cell elements. Is it not therefore more reasonable to accept as a provisional hypothesis the view that yolk formation is a very intricate interaction of the metabolic functions of plasmosome, mitochondria and Golgi apparatus, a hypothesis which, while unifying the data, is perfectly compatible with the observed transformation of deutosomes (nuclear emissions), chondriosomes and dictyosomes in individual cases? It is in no way implied that Schaxel's account of nuclear emission in the vitellogenesis of hydroids is accepted; for a criticism of this work, Beckwith's paper (20) may be consulted.

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* Saline corrosive 2½ hours, aq. dest., osmic acid 2 per cent. 10 days.

Synopsis.

(1) Nuclear organisation in the germ cells of *L. depressa* has been investigated; emphasis is laid especially on two points: (*a*) the importance of the study of premeiotic kinetic phases to the interpretation of synapsis; (*b*) the independence of the plasmosome as a nuclear constituent from the chromatinic organisation.

(2) The diploid complex in the male germ cells of *L. depressa* consists of twenty-three approximately equal chromosomes, that of the female consisting of twenty-four.

(3) The chromosomes of the premeiotic mitoses become more curved and display greater individuality *inter se* as they pass undivided to the poles of the spindle; in the telophase they spin out into finely granular loops which initially occupy a polar disposition; they become increasingly attenuated in the spirophase (reticulate stage), so that individually they cannot be identified: in the prophase the reverse process occurs; they are first recognisable individually as convoluted threads, contracting into beaded loops which become abbreviated to form longitudinally split arc-shaped chromosomes.

(4) The failure of modern cytological research to confirm the existence of Flemming's continuous spireme makes it imperative for those who advocate telosynapsis in animal forms to postulate some means whereby terminal union of homologous chromosomes can be effected in a manner consonant with the observed data respecting the premeiotic telophase and the synaptic processes. Zoological cytologists who have adopted this view have not yet offered such information.

(5) Parasynapsis can be observed in the oocytes of Libellula: the method conjunction is probably the same in the male germ cells.

(6) The transverse axis of the tetrad in the first spermatocyte mitosis corresponds with the longitudinal split in the diplotene stage; the X-element is segregated in the second reduction division.

(7) The behaviour of the double nucleolus in Libellula has been investi-

gated, and provides no sanction for the view that the plasmosome is in any way connected with the chromatinic organisation of the nucleus: its relation to yolk formation is indicated.

(8) Data relating to the transmission of material from nucleus to cytoplasm are discussed in their bearing on "chromidia" and secondary "nuclei"; it is submitted that the origin of the latter in no way involves a revision of the view that the chromatin organisation of the nucleus retains its integral continuity throughout the growth phase.

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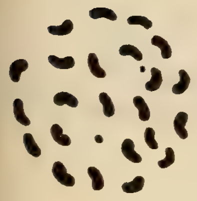
EXPLANATION OF PLATES.

Fig. 1.—Spermatogonial metaphase: 23 chromosomes and two granules ? nucleolar in origin. 2.—Do., anaphase. 3–5.—Telophase: *p*, plasmosome. 6.—Spirophase. 7–10.—Prophase. 11.—Spermatocyte: Leptotene stage, X, X-chromosome. 12.—Contraction phase. 13.—Do., pachytene. 14–15.—Diplotene stage. 16.—Dissolution of bouquet. 17–18.—Genesis of tetrads. 19–22.—Heterotype mitosis. 23–25.—Second reduction division with 11 and 12 chromosomes. 26.—Oogonial prophase. 27.—Metaphase with 24 chromosomes. 28.—Oocyte: leptotene stage. 29.—Zygonema. 30.—Maximal contraction. 31.—T.S., late pachytene stage. 32.—Diplotene stage (post-synaptic). 33–35.—Genesis of pro-tetrads and inception of the diffuse stage. 36.—Oocyte in growth period with double nucleolus (*p*), nucleus-like intranucleolar oxyphil body (*o*), chromatic network (*cr*), deutosomes in nucleus and cytoplasm (*d*). *Flemming.* 37.—Do., showing formation of the oxyphil body. *Flemming.* 38.—Oocyte at inception of growth period with yolk nucleus or mitosome. *Mann-Kopsch.* 39.—T.S. older egg, mitochondrial area (*m*). *F.W.A.*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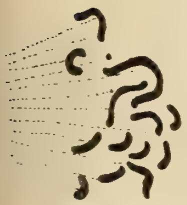
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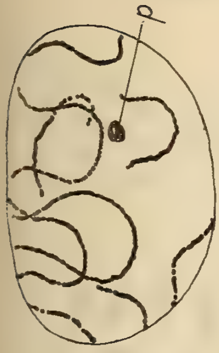

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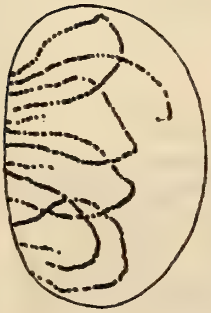
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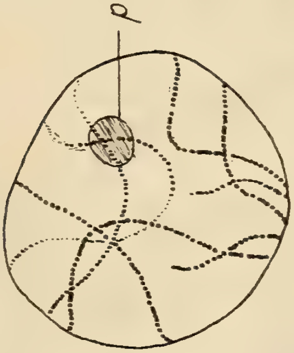

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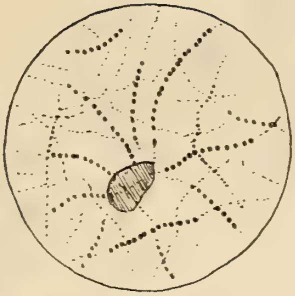
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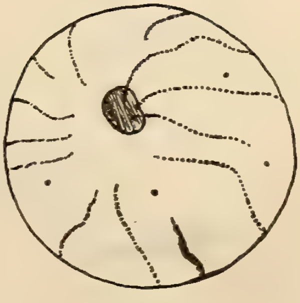
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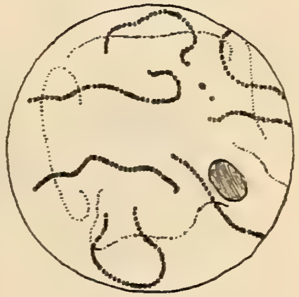
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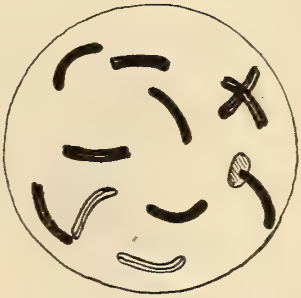
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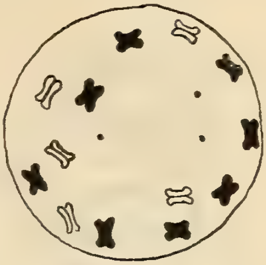
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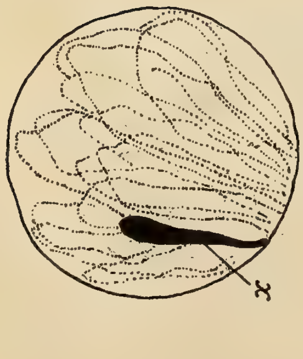

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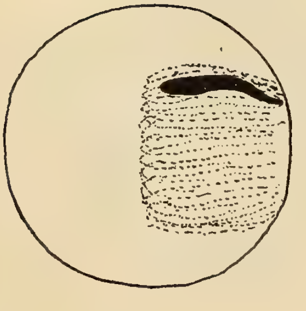

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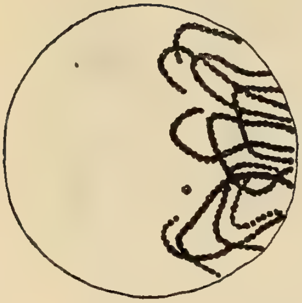

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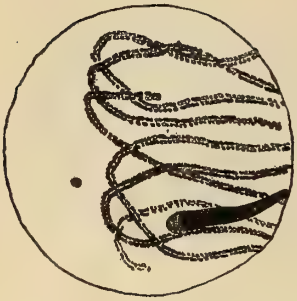

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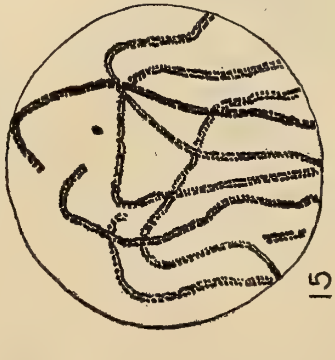

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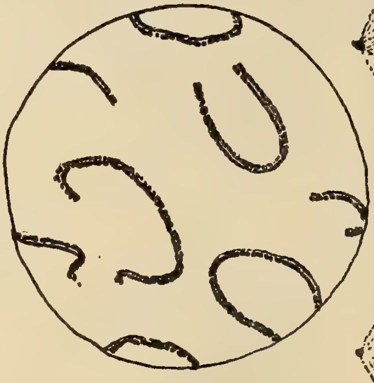

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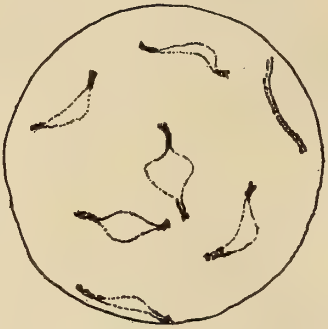

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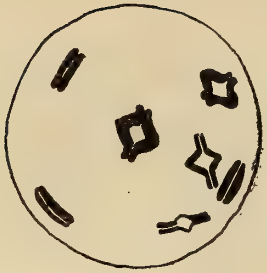

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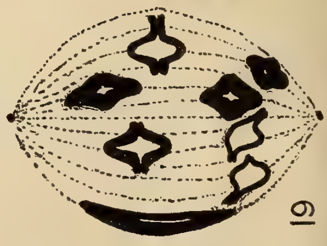

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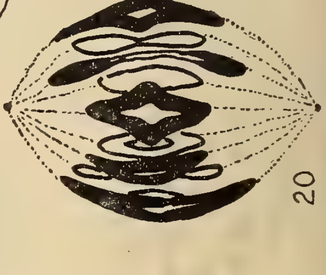

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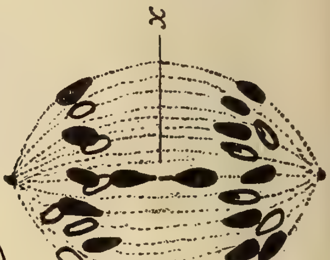

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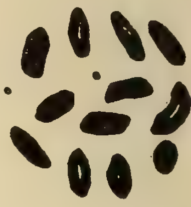

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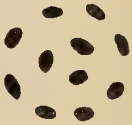

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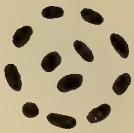

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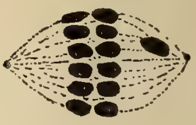

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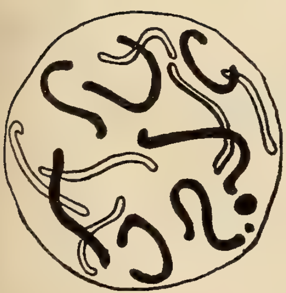

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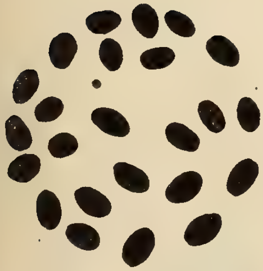

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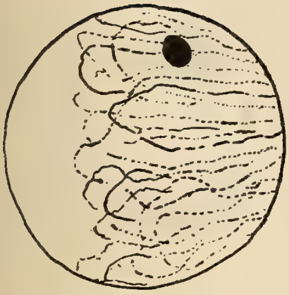

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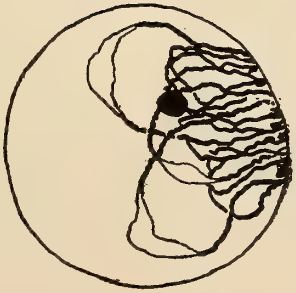

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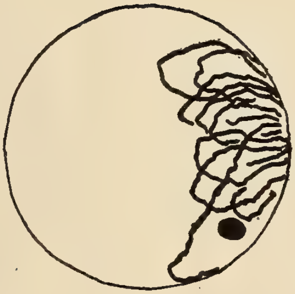

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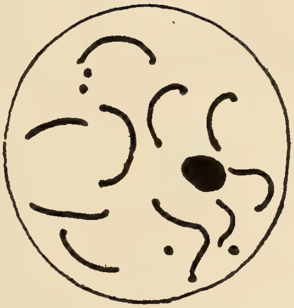

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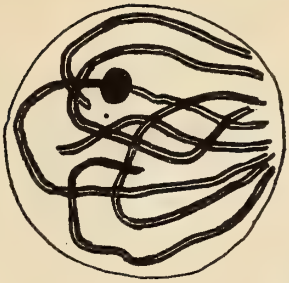

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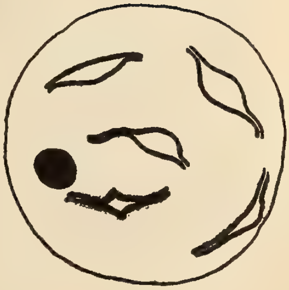

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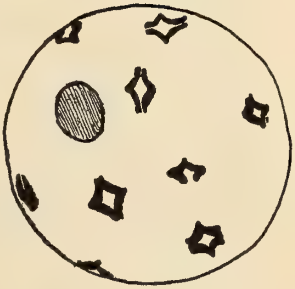

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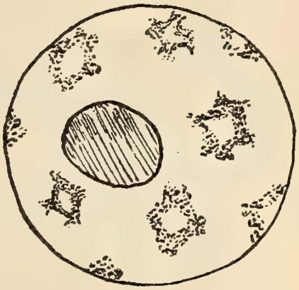

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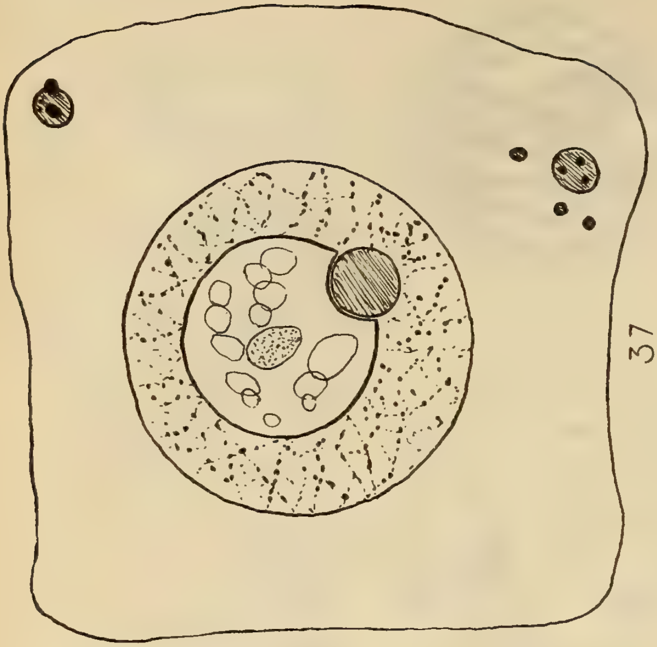
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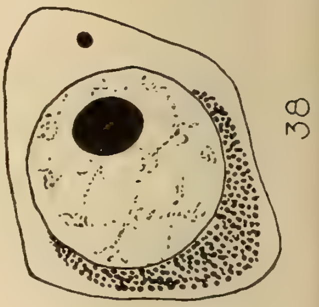
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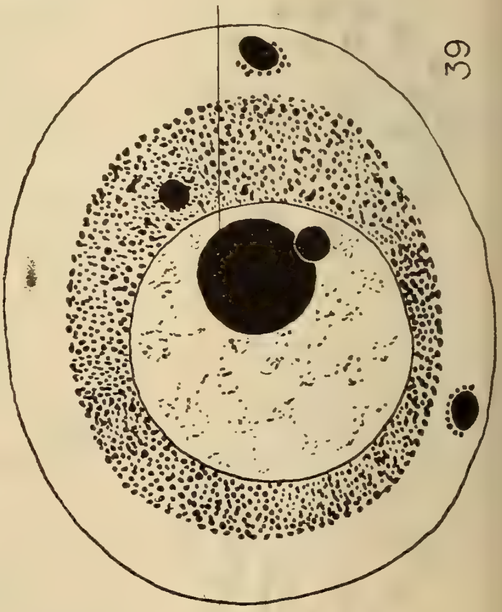
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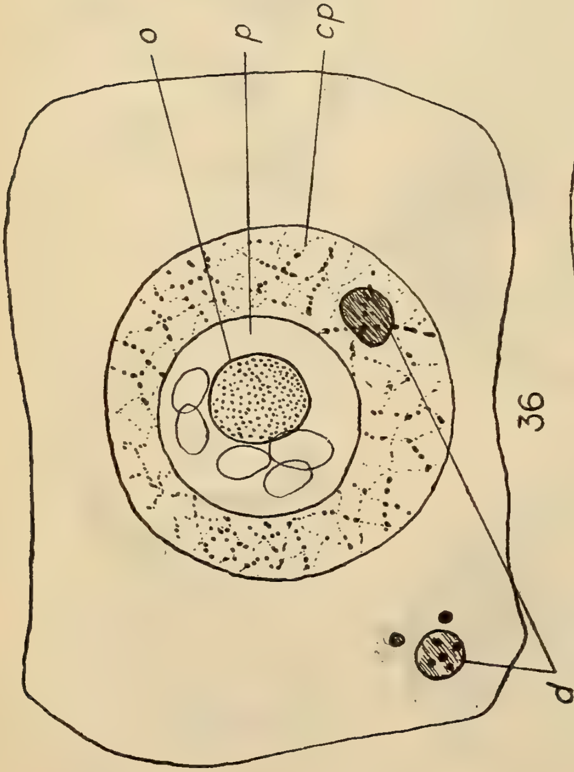
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Cellular Immunity: Observations on Natural and Acquired Immunity to Cobra Venom.

By J. A. Gunn and R. St. A. Heathcote.

(Communicated by Prof. Sherrington, F.R.S. Received October 16, 1920.)

(From the Pharmacology Laboratory, Oxford.)

Introductory (J. A. Gunn).

While the properties acquired by the serum of an animal as the result of immunisation to a toxin of the bacterial type have been examined with an exhaustive minuteness, little of the now vast literature on immunity has concerned itself with the cellular as opposed to the humoral aspect of immunity. Little, and less that is certain, is known of the changes that immunisation produces in living cells other than the white blood corpuscles. The part played by these cells in immunity processes, so fruitfully studied by Metchnikoff and others, is responsible by itself for an extensive literature which cannot be dealt with here. Accurate information is still wanting in regard to the part played by other living cells in the acquisition and retention of immunity. One reason for this is that investigation has been confined too exclusively to the blood. This has been partly and justifiably due to the diagnostic, therapeutic, and other importance of immune sera; also, perhaps, to the fact that the technique of blood investigation is more easy and generally familiar than the technique necessary to deal with other tissues. Another reason, no doubt, is that there are relatively few toxins which produce true immunity that lend themselves to the kind of investigation adopted in the experiments to be described.

Without attempting the task, here unnecessary, of making a complete survey of the literature on cellular immunity, I wish to state briefly the present state of knowledge in regard to certain factors which have been investigated as explanatory of (*a*) natural, and (*b*) acquired immunity.

(a) *Natural Immunity.*—It is now generally believed that natural immunity either to drugs, poisons, or toxins is seldom, if ever, due to the presence of antitoxin in the blood of the immune animal. For example, Calmette and Delearde (1) found that there is no antitoxic substance in the blood of reptiles capable of explaining the relative natural immunity which they possess to venom; and similarly, that the sera of the fowl and tortoise, which resist high doses of abrin, are completely devoid of antitoxic power. Though they did find that the sera of the mongoose and hedgehog (which both show high immunity to venom) possess antitoxic properties, those properties are slightly developed, and do not accord with the degree of immunity. They therefore concluded that there is no correlation between the naturally refractory state which certain animals possess and the antitoxic power of their sera to the toxins to which they are insensitive.

Camus and Gley (2) found that the serum of the hedgehog possesses no antitoxic action against eel serum, though this animal withstands a dose twenty times greater than the rabbit. Pettit (3) found that the relative immunity of the rat to diphtheria toxin is not due to an antitoxin in the rat's serum.

It may be stated generally, as the result of these and many other observations on a variety of poisons and toxins, that, though isolated cases are known (4) where the serum possesses unusual antagonising powers, natural immunity is not generally to any extent, and probably never completely, due to the presence of antitoxins in the blood.

It has generally been assumed, therefore, from this that natural immunity is due to a specific insensitiveness of the animal cells to the action of the toxin. Positive proof of this assumption has not so often been brought forward. One aspect of it has been carefully examined by Camus and Gley. They found that the red blood corpuscles of the hedgehog (2), as well as of other animals, *e.g.*, the toad, pigeon, and bat (5), which possess a relative natural immunity to eel serum, show an increased resistance to the hæmolytic action of this toxin. Those experiments seemed to establish a relationship between natural immunity and cellular resistance, so far, at least, as the erythrocytes were concerned, but Camus and Gley later found that the red blood corpuscles of the marmot also (6), and of the cat (7), show a high resistance to the hæmolytic action of eel serum, though both animals are extremely sensitive to the toxic action of the serum. The relationship was therefore not constant. They therefore concluded that natural immunity is a complex phenomenon, and that the resistance manifested by one tissue does not imply a similar resistance of the other tissues of the animal towards a particular toxin.

In regard to the natural immunity of the rat to strophanthus (8), I was able to show a clear quantitative relationship between immunity and cellular resistance.

I found that the minimum lethal dose of strophanthus per kilogramme for the rat is thirty times the minimum lethal dose per kilogramme for the rabbit. When the excised hearts of rats and rabbits are artificially perfused with solutions of strophanthus—a procedure which previously removes the serum—it was found that it requires about thirty times as strong a solution to arrest the rat's heart as is required to arrest the rabbit's heart in the same time.

The conclusion was drawn that the congenital tolerance of the rat to strophanthus is due chiefly, if not entirely, to an insusceptibility of the heart of this animal to the action of strophanthus. This poison was particularly suitable for establishing such a quantitative relationship—if it existed—as arrest of the heart is the primary cause of death in strophanthus poisoning.

Two subsidiary points were established which find a parallel in the experiments to be described in this paper. In the first place, the ratio of 30 : 1—the concentration necessary to arrest the rat and rabbit heart respectively—held good only for such lower concentrations as arrest the heart slowly; with high concentrations, the ratio was nearer 2 : 1. The theoretical bearing of these results is discussed in the original paper. In the second place, though the rat's heart showed a specific insusceptibility as compared with the rabbit's heart to the action of strophanthus, comparable to the difference in the lethality of strophanthus for the intact animals, no difference was found between rat's and rabbit's red blood corpuscles in their resistance to strophanthus hæmolysis.

Many years ago, Kanthack (9) made a careful quantitative study of the relationship between the lethality and hæmolytic action of cobra venom for a variety of cold- and warm-blooded animals, and came to the conclusion that "there is no absolute relation between the hæmolytic reaction of the blood of an animal to cobra poison and the resistance of the animal itself to the poison."

We have in these papers a series of observations, made, with different poisons, to determine whether any relationship exists between cellular resistance and natural immunity. From its convenience and familiarity as an experimental cell, it was natural that the red blood corpuscle should be employed in cases where the poison acted on it. It is however important to realise that, alike with eel-serum, cobra venom, and strophanthus, no consistent relation, if any, was found to exist between natural immunity of the animal and resistance of its erythrocyte to hæmolysis. On the other hand, in the case of strophanthus, when the heart was employed, the existence of a quantitative relation between immunity of the animal and resistance of the heart was clearly demonstrable. The significance of this, especially the failure of the red cell as a reliable index of cellular immunity, will be discussed later.

(b) *Acquired Immunity.*—It is of perhaps greater interest to determine whether, when an animal is actively immunised to a toxin, the cells of the animal become more resistant to the toxin. Relatively few observations seem to have been made on this point, though it might afford an explanation of many problems, for example, why a high degree of immunity may exist with the presence of a feeble antitoxin in the blood, and why immunity should in some cases persist after antitoxin has disappeared from the blood. There can be no doubt that this is a question of fundamental importance which demands a larger share of attention than has so far been bestowed upon it.

Camus and Gley (10) found that the washed red corpuscles of a rabbit immunised to eel serum showed augmented resistance to the hæmolytic action of this serum. No quantitative determinations are given but it is stated that the increased resistance was sometimes very slight.

Kossel (11) stated that the red cells of rabbits immunised to eel serum, and carefully freed from serum, became more resistant to the hæmolytic action and in proportion to the grade of immunity; but he gives no details of the experiments.

Later Camus and Gley (12) found no increased resistance of the red cells in rabbits after prolonged immunisation to eel serum, but in an animal rapidly immunised (by four injections in six days) they found that some of the corpuscles were not hæmolysed even by high concentrations—an effect due, they suppose, to the fact that the less resistant corpuscles have been destroyed.

On the other hand, Cushny (13) found that the blood of a rabbit immunised to 5000 times the minimum lethal dose of ricin still showed agglutination like that of a normal rabbit, only it appeared " even somewhat more sensitive." Jacoby (14) who immunised a goat for eleven months and at intervals tested the resistance of the red cells, found them still agglutinable, when free from serum, even in the highest stages of immunity. He pointed out that the possibility of cellular immunity of the red cells occurring in still higher stages of immunity was not excluded.

Calmette (15) stated clearly, though so far as I can find he did not subsequently publish details of his experiments, that the red blood corpuscles of an animal, immunised to cobra venom and which yielded a very antitoxic and antihæmolytic serum, were still perfectly hæmolysable after being freed from serum, by feeble doses of venom to which had been added a little normal serum heated to 62°.

So far as concerns the red blood corpuscles, therefore, no acquired cellular immunity has been found except to eel serum; and apparently with ricin and cobra venom observers have hesitated to commit themselves to the paradoxical result that the red cells may become actually more sensitive.

In regard to acquired cellular immunity of other tissues, the results are not very convincing. Gley (16) found that the toxicity of eel serum for the rabbit is ten times greater when injected into the cerebro-spinal fluid than when injected intravenously. When a rabbit was immunised the minimum lethal dose by cerebro-spinal injection was practically unaltered, and he therefore concluded that the central nervous system does not acquire immunity to eel serum, or at least only a very feeble immunity.

Gley (17) came to the same conclusion with torpedo serum, namely, that there was no cellular immunity as far as the central nervous system was concerned.

These experiments were of the same type as those of Roux and Borrel (18) who found that rabbits immunised against tetanus are as easily as before made tetanic by injections into the brain itself. Such experiments are ill adapted to prove the point at issue, because, though relatively small amounts of toxin are injected into the brain or cerebro-spinal fluid, they are injected in high concentration compared to that which arrives at the central nervous system when the toxin is diffused through the blood.

Gley and Pachon (19) prosecuted further researches on this problem to determine whether there was any cellular immunity developed in the heart when an animal is immunised to toxic sera. Their experiments were inconclusive. Indeed the toxins employed were not suitable for deciding the point. It required 1 to 4 per cent. of eel serum and 10 to 20 per cent. of torpedo serum to produce even doubtful effects on the heart. Cushny and Gunn (20) have shown more definite effects with lower concentrations of horse serum, so that it is doubtful if the serum of the eel or torpedo has any specific toxic action on the heart.

So far as acquired immunity is concerned these observations leave the question still undecided. According to Camus and Gley and Kossel, in an animal immunised to eel serum the red cells, freed from antitoxin, may show an increased resistance to the hæmolytic action of the serum, but a similar effect was not found by Cushny or Jacoby with ricin or by Calmette with cobra venom, and no convincing proof was forthcoming of an increased cellular immunity of the heart or central nervous system occurring in the process of immunity.

It was my intention to proceed, after completing the experiments described with strophanthus, to determine by similar methods whether when an animal is immunised to a particular toxin, the tissues of the animal freed from the serum acquire an increased insusceptibility to the action of the toxin. For this purpose it was necessary to employ a toxin of the bacterial type which produces genuine immunity with antibodies in the serum. Of such toxins among the most convenient to work with, for well-known reasons, are either the snake venoms or one of the vegetable toxins, such as ricin.

I have at various times begun such experiments with such samples of snake venom as I could procure, but have been unable to complete them from want of a sufficient amount of a sufficiently active venom. All the experiments have, of course, to be completed with the same sample of venom. I also tried ricin, which was from one point of view more suitable, because in contradistinction to what is true of snake venoms it is extremely easy to immunise rabbits to ricin. But unfortunately I found that ricin produced no effect on the isolated heart or intestine of the rabbit, even in a concentration of 1 in 5000, although the minimum lethal dose of it for this animal was 0·05 mgrm. per kilogramme. It was clearly unsuitable for this kind of investigation. I found, however, that the red blood corpuscles of an animal immunised to ricin are *more* easily agglutinated than are the red blood corpuscles of a normal rabbit.

Cushny, as has been pointed out, had found that the blood of a rabbit immunised to 5000 times the minimum lethal dose of ricin still showed agglutination like that of a normal rabbit, only it appeared even somewhat more sensitive. As a matter of fact the increased sensitiveness is well beyond experimental error. For example, I found by parallel experiments that the washed corpuscles of a rabbit immunised to twenty minimum lethal doses of ricin were agglutinated by 0·0005 per cent. of ricin as compared with 0·002 per cent. required to agglutinate washed corpusles of a normal rabbit. Those observations suggested to me a doubt as to whether the red cells could be a reliable index of acquired cellular immunity, presuming that such a condition occurred. As has been pointed out above, they have certainly not been found to be so in natural immunity, and *a priori* it is less likely that they should be so in acquired immunity for these reasons. The mammalian red cell is a cell that has lost its nucleus. It is probably not a living cell in the ordinary sense at all but a mere box with a transitory existence. It is difficult to imagine in what way the *formed* red cell could develop an increased resistance in the process of immunisation. It is true that a more resistant cell might be manufactured in the bone marrow and so be distributed into the blood. But on the whole it seemed unwise to employ a cell that is, both histologically and in regard to its passing existence, different from most cells of the body as an index of what occurs, in the process of immunisation, to the cells of the body generally.

I am not suggesting—indeed the experiments of Camus and Gley and Kossel point otherwise—that at no period in the course of immunisation will an increased resistance be found in the red cells if the other cells of the body acquire it. But the experiments to be described, I think, render the red cells still further suspect as a test of cellular immunity. The peculiar character of the red cell has not been sufficiently realized and, it may be, has been the means of preventing work on cellular immunity, for though it is the test object that would first suggest itself, it is the most misleading guide to the condition of the cells generally.

In 1912, as a preparation for the scheme of experiment now undertaken, I investigated in greater detail than had hitherto been done the action of cobra venom on isolated mammalian tissues. I came to the conclusion (21) that cobra venom contains some substance having an action closely approximating to a sympathomimetic action. Cushny and Yagi later (22), working with a different sample of venom, obtained results differing in certain particulars, especially in the fact that they obtained no inhibitory action on smooth muscle and no stimulation of secreting glands such as I had obtained; and they therefore came to the conclusion that cobra venom does not contain any substance of sympathomimetic action. In regard to a difference of results of this nature, it is well to keep in mind that snake venoms are, so far as is known, substances of complex nature and presumed to contain a variety of toxins of varying stability. Complete concordance of results in regard to primary action cannot, therefore, be expected with different samples of venom, collected in different ways, and kept under different conditions and for different times. It is, however, interesting to find that Abel and Macht (23) have discovered in the skin secretion of the toad two substances (*a*) a substance having an action like digitalis, and (*b*) adrenaline. Cushny and Yagi found that cobra venom acted rather like digitalis, while I found that it acted rather like adrenaline. Perhaps the question as to whether both may not occur in cobra venom, as in toad poison, ought to be left open. The question ought to be decided by determining the action of a perfectly fresh venom and by examining the glands for the chromaphil reaction. In any case the venom I have now used resembles more nearly that employed by Cushny and Yagi. Especially, this sample of venom caused a marked rise of tone in the isolated rabbit's intestine; but the addition of a minute trace of adrenaline (1 in 30,000,000) prevented this rise of tone even with high concentrations of venom and produced an effect exactly resembling that previously found by me.

Observations on Natural and Acquired Immunity to Cobra Venom. (Gunn and Heathcote.)

The Congenital Immunity of the Cat to Cobra Venom.

It has long been known that animals show different degrees of susceptibility to the toxic action of venoms, and especially that the cat shows a high degree of resistance as compared with rodents. Thus Fraser (24) in 1896 showed that the minimum lethal dose of cobra venom for the cat is thirty times that for the rabbit, and Fraser and Gunn (25) that the minimum lethal dose of *Sapedon hæmachates*—another colubrine venom—is for the cat fifteen times that for the rabbit. This comparative tolerance of the cat is most marked in the case of colubrine venoms, but it is also true to a certain extent of viper venoms, for Fraser and Gunn (26) found that the ratio of lethality for the cat and rabbit in the case of Echis venom was 9 : 1.

Minimum Lethal Dose for Rabbit and Cat.

The minimum lethal dose of the venom we used was found to be for the rabbit 0·0012 grm. per kilogramme and for the cat 0·025 gramme per kilogramme. Calculated per kilogramme, therefore, the minimum dose required to kill the cat is twenty times greater than that required to kill the rabbit. As this relation is similar to that found by Fraser and others, it is not necessary to give the experiments in detail.

The following experiments were made to ascertain whether or not, corresponding to this relative natural immunity of the intact animal, the tissues of the cat are less susceptible to the action of the venom than are the tissues of the rabbit. With this end in view, experiments were made on the heart, intestine, and red blood-corpuscles.

(a) *Heart.*

For perfusing the heart and for recording the flow through the coronary vessels, the heart-perfusion apparatus (27) and syphon-recorder (28), described by one of us were employed. As the perfusing solution, Locke's solution without glucose was used. The hearts were perfused for at least twenty minutes before the venom solution was tried, so that all the serum was removed from the heart first.

Cobra venom, if in sufficient concentration, kills the heart by arresting it in the systolic position. The preliminary effects are somewhat variable, there being sometimes a primary enfeeblement of systolic contraction, occasionally a small and short-lasting augmentation or acceleration.

Table I.—Comparison of the Action of Cobra Venom on the Perfused Heart of the Rabbit and Cat.

Strength of solution.	Rabbit.	Cat.
1 in 5,000	Systolic arrest in 8 minutes	Systolic arrest in 7 minutes.
,, 10,000	,, ,, 8 ,,	—
,, 20,000	,, ,, 8 ,,	—
,, 50,000	,, ,, 10 ,,	Systolic arrest in 11 minutes.
,, 100,000	,, ,, 15 ,,	,, ,, 17 ,,
,, 200,000	,, ,, 13 ,,	,, ,, 31 ,,
,, 400,000	,, ,, 15 ,,	,, ,, 34 ,,
,, 600,000	,, ,, 20 ,,	Not arrested in 80 minutes.
,, 800,000	,, ,, 34 ,,	,, ,, 80 ,,
,, 1,000,000	—	,, ,, 105 ,,
,, 2,000,000	Systolic arrest in 73 minutes	—

This table shows clearly that the heart of the cat is much less susceptible than that of the rabbit to the action of the venom. This does not appear with high concentrations, *e.g.*, there is no appreciable quantitative difference in the action on the rabbit and cat heart in concentrations ranging from 1 in 5000 to 1 in 100,000. With lower concentrations, there is an unmistakable difference in the susceptibility of the hearts of the two species of animals, whether this be judged from the time required to produce a given effect, or from the minimum concentration required to kill the heart in systole. Thus, 1 in 200,000 arrests the heart of the cat in 31 minutes, whereas half that strength (1 in 400,000) arrests the rabbit's heart in half the time. Or a strength of 1 in 200,000 produces practically the same effect on the cat's heart as one of 1 in 800,000 on the rabbit's. Judged from this standpoint, the immunity of the cat's heart is to that of the rabbit's as 4:1. Further, a solution of 1 in 2,000,000 arrests the rabbit's heart in 73 minutes, whereas a solution of 1 in 800,000 does not arrest the cat's heart in 80 minutes.

In figs. 1 and 2, the effects of 1 in 600,000 are shown on the hearts of the rabbit and cat. The rabbit's heart is completely arrested in 20 minutes, whereas the cat's heart is not markedly affected in 70 minutes.

It is clear, therefore, that the cat's heart, as compared with the rabbit's, possesses in itself a tolerance to cobra venom. A larger number of experiments would be required to give an exact numerical ratio to the difference in susceptibility, if, indeed, it would even then be possible in such experiments to give exactly such a ratio. For the present purpose, it is sufficient to establish the fact that the difference is pronounced, and that, judged from an arbitrary end-point, it requires four times as strong a solution to produce a given effect on the cat's heart as is required to produce that effect on the rabbit's.

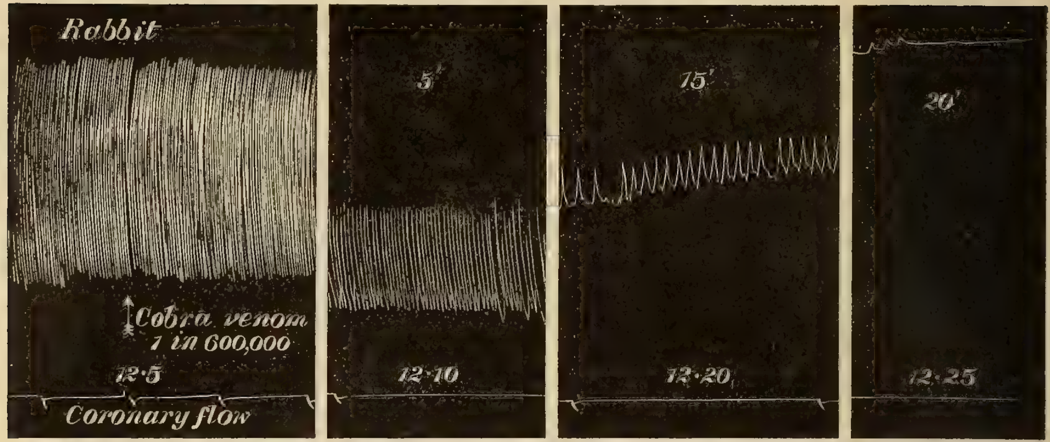

Fig. 1.—Isolated rabbit heart perfused with cobra venom, 1 in 600,000.

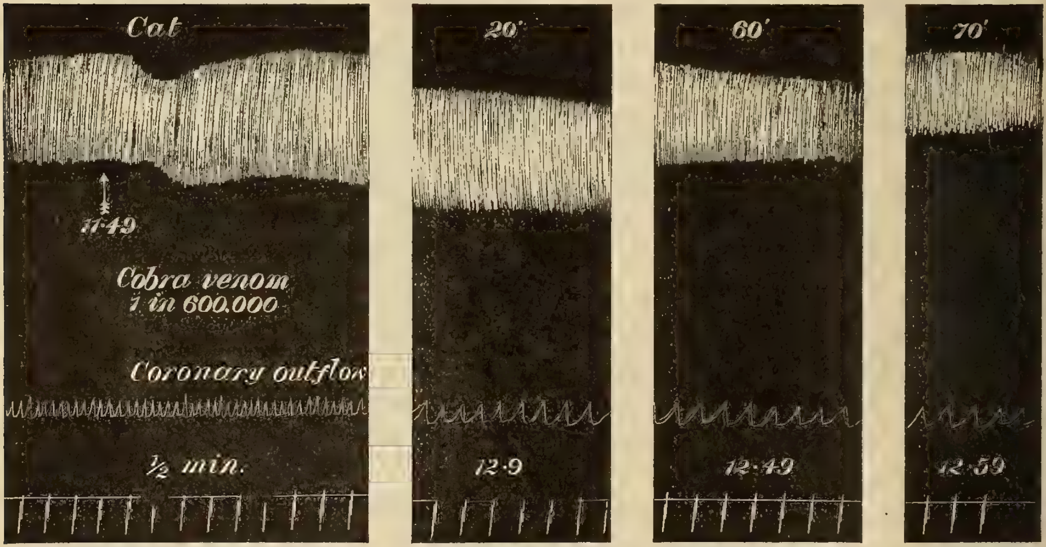

Fig. 2.—Isolated cat heart perfused with cobra venom, 1 in 600,000. Showing that whereas the rabbit heart is completely arrested in systole in 20 minutes, the cat heart is not markedly affected in 70 minutes by the same strength of solution.

(b) *Coronary Blood-vessels.*

When either the rabbit's or the cat's heart is perfused with solutions of cobra venom, the flow through the vessels is markedly diminished as soon as the ventricle goes into systolic contraction. This effect occurs with other substances, which produce rigor of the heart muscle. The flow is stopped by compression and indirect occlusion of the vessels, as the result of the contracture of the heart muscle surrounding them.

The chief point of interest lies in the effect of the venom on the coronary flow prior to the onset of rigor of the heart. In the rabbit, the flow always diminishes from the beginning, independently of the alteration in the heart beat. The effect is therefore no doubt a direct action on the arterial wall, similar to that which occurs with other types of smooth muscle, and which will be dealt with in the next section. In the cat, on the other hand, there is generally a preliminary increase in the coronary flow, sometimes to the extent of over 50 per cent., which may last for several minutes. This, again, is independent of changes in the heart's contractions, and is therefore probably due to the venom causing a preliminary dilatation of the coronary arteries. The effect is not constant, and in its nature and occurrence resembles the effect produced by the venom on the smooth muscle of the intestine, which in the cat is sometimes relaxed by the venom.

The conclusions we draw from these experiments are (1) that the heart muscle of the cat is much less susceptible to the toxic action of the venom than is the heart muscle of the rabbit, and (2) that there is a qualitative difference in the reaction of the coronary arteries of the two species of animals, those of the cat being usually at first relaxed by the venom, those of the rabbit always contracted from the beginning.

(c) *Intestine.*

A large number of experiments were performed to compare the effects of cobra venom on the intestine of the rabbit and of the cat. These experiments were performed by the now familiar method of Magnus, sections of the small intestine, about 2 cm. in length, being suspended in a bath of oxygenated Locke's solution at 37° C. The bath contained 50 c.c., and the venom was added in solution in known amounts, so that the resulting concentration of it could easily be calculated.

For the purpose of an investigation like the present, the intestine does not give such reliable quantitative results as the perfused heart. When the heart is perfused, the venom acts uniformly throughout the substance of the heart muscle, whereas when the venom is added to the solution surrounding the suspended intestine, the action to begin with is a superficial action. Substances which act on the nerve-endings, like pilocarpine or adrenaline, exert their actions almost instantaneously on the isolated intestine when the effects are recorded in the manner here adopted. With substances that act on the muscle, like cobra venom, which is also probably slowly diffusible, there is a definite delay in the production of the full effect of a certain concentration on the isolated (non-perfused) gut, a delay due to the fact that the venom reaches superficial muscle fibres before deeper ones.

This fact is mentioned only because time effects and possibly even quantitative effects of the venom cannot be gauged with the same accuracy on the intestinal muscle as on the perfused heart. There is also more irregularity in the quantitative results on different segments of intestine than one obtains in the perfused heart. This is true of other substances, *e.g.*, adrenaline, as well as of cobra venom.

The following summary, however, shows that, in spite of these drawbacks, the effects of cobra venom on the intestine of the cat differ sufficiently, and sufficiently uniformly from its effects on the intestine of the rabbit to allow of conclusions being drawn from the experiments.

With the rabbit's intestine, a solution of 1 in 10,000, 1 in 20,000, and 1 in 25,000 caused tonic contraction of the gut with arrest of the movements in a few minutes, and 1 in 50,000 stopped the movements in the same fashion in half an hour. With the cat's intestine, a solution of 1 in 5,000 caused a slight preliminary rise of tone, which gradually passed off and the movements ceased in a position of relaxation in 25 minutes. 1 in 10,000 caused relaxation of the gut, sometimes marked, but did not arrest the movements in half an hour; 1 in 20,000, 1 in 25,000, and 1 in 50,000 caused slight relaxation, but had very little effect on the amplitude of the segmentation movements, which were still vigorous at the end of half an hour. The experiments with each concentration were repeated twice.

In figs. 3 and 4 is shown a comparison of the differences on the rabbit and cat intestine of cobra venom 1 in 10,000.

These experiments therefore show that there is both a quantitative and a qualitative difference in the action of the venom on the intestine of the rabbit as compared with that of the cat. The quantitative difference consists in the fact that much higher concentrations are required to kill the intestine of the cat than to kill that of the rabbit, and the qualitative difference in the fact that the type of effect differs somewhat in the two animals. In the rabbit the venom always causes arrest of the intestinal movements in systole, while in the cat the venom usually produces increased relaxation.

Red Blood Corpuscles.

Experiments were made on washed red blood corpuscles of the cat and rabbit. A 4 per cent. suspension, twice centrifuged and washed, was used in each case. The saline solution used was 0·9 per cent. sodium chloride in distilled water. The venom solution was made by dissolving the venom in the saline solution. This venom solution was divided into two parts, one of which was used for the experiment on rabbit's corpuscles, the other for that on cat's corpuscles. This obviated experimental errors in weighing and differences in

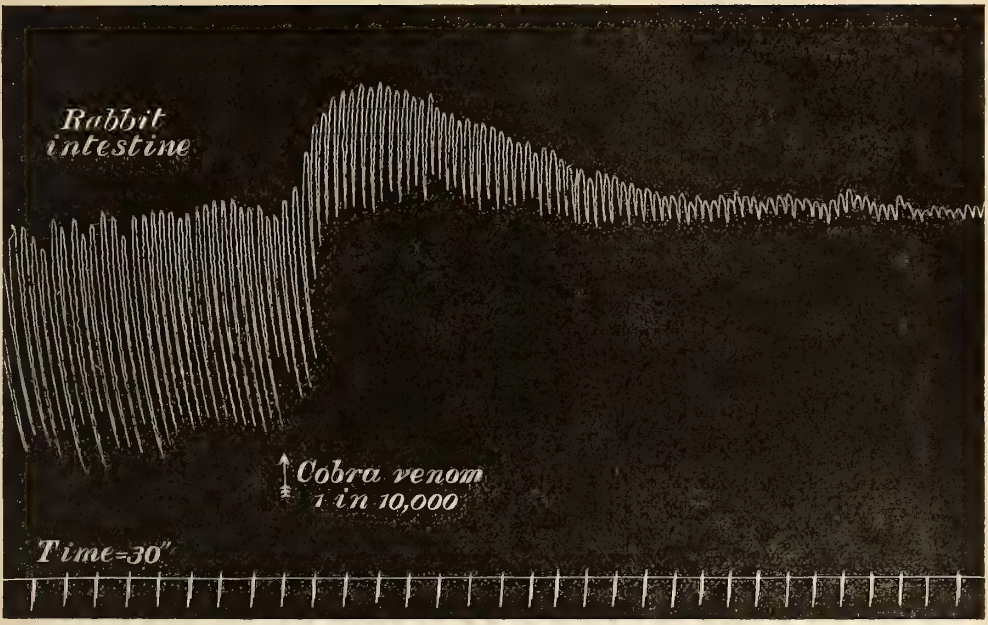

Fig. 3.—Isolated rabbit intestine.

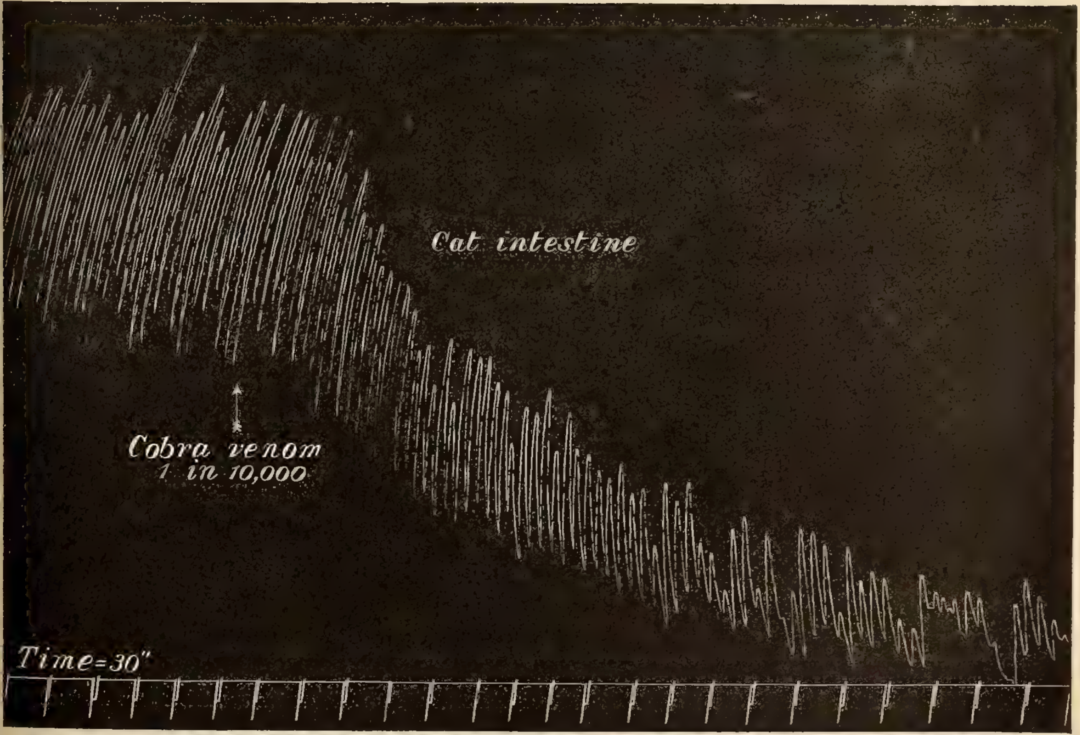

Fig. 4.—Isolated cat intestine. Showing difference in the effects of cobra venom, 1 in 10,000.

the composition of the venom. The times of centrifuging and keeping the blood of the two animals were exactly the same, so that the experiments were as nearly as possible paralleled.

The following Table gives a skeleton of the experiments performed to determine the minimum hæmolytic dose of the venom for rabbit's and cat's corpuscles respectively :—

Table II.—Scheme of Hæmolytic Experiments.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
	C.V. 0·1 per cent.					C.V. 0·01 per cent.				
Blood suspension, c.c. ...	0·25	0·25	0·25	0·25	0·25	0·25	0·25	0·25	0·25	0·25
Cobra venom, c.c.	0·25	0·2	0·15	0·1	0·05	0·25	0·2	0·15	0·1	0
Saline solution, c.c.	0	0·05	0·1	0·15	0·2	0	0·05	0·1	0·15	0·25
Concentration of venom per cent.	0·05	0·04	0·033	0·02	0·01	0·005	0·004	0·0033	0·002	0

The tubes, therefore, each contained the same amount of corpuscles (as far as can be judged without actually counting them), the same concentration of sodium chloride, and the same amount of total solution, but differed only in their venom-content.

Two parallel experiments were done in this way with rabbit's and cat's corpuscles. The tubes were incubated at 35° C. In 3½ hours the cat's corpuscles were hæmolysed completely in tubes 1, 2, 3, and 4, and just not completely in 5. The rabbit's corpuscles were also completely hæmolysed in tubes 1, 2, 3, and 4 and there was a trace of hæmolysis in 5. There was no trace of hæmolysis in any of the other tubes. The tubes were re-shaken, allowed to stand at room temperature overnight and read 15 hours later. The cat's corpuscles were then found to be completely hæmolysed in the first six tubes and the rabbit's in the first five. Otherwise stated, in 3½ hours cat's corpuscles are completely hæmolysed by a concentration of 0·02 per cent. and almost completely hæmolysed by 0·01 per cent. Rabbit's corpuscles are completely hæmolysed by 0·02 per cent. and slightly hæmolysed by 0·01 per cent. In 18½ hours cat's corpuscles are completely hæmolysed by 0·005 per cent. and rabbit's corpuscles by 0·01 per cent., under the same temperature conditions. There is, therefore, only a slight difference between the susceptibility of the cat's and rabbit's corpuscles to the hæmolytic action of the cobra venom, the cat's corpuscles being hæmolysed by slightly *lower* concentrations.

The conclusion to be drawn from these experiments is that, though the cat possesses, as compared with the rabbit, a high degree of congenital immunity to cobra venom, the red blood corpuscles of the cat are less resistant to the hæmolytic action of the venom than are the red blood corpuscles of the rabbit.

Acquired Immunity to Cobra Venom.

The object of these experiments was to determine whether, when an animal is actively immunised to cobra venom, the tissues of the animal, free from the serum, acquire increased resistance to the venom. For this purpose we compared quantitatively the effects of the venom on the heart, intestine and blood corpuscles of an immunised animal with its effects on the same organs of normal unimmunised animals.

The difficulty of immunising rabbits to cobra venom is well known and it is not necessary for the purposes of this paper to deal with the question. It is sufficient to state that, of twelve rabbits, which we began to immunise in February, 1920, only one survived immunisation to the degree of tolerating 10 minimum lethal doses. This rabbit received the injections intravenously into the marginal veins of the ear. The doses were increased very gradually. It weighed 1420 grm. on February 7, 1920, the day on which it received its first venom injection, and on June 6, 1920, when it received its last injection of 10 M.L.D., it weighed 2580 grm. This last injection caused a fall of weight on the following day to 2450 grm. but the animal recovered its normal weight by June 10 when it was killed and its isolated organs used for experiments.

The following experiments were performed:—

(a) *Heart.*

In Table I was shown the effect of the venom we were employing on the isolated hearts of normal rabbits. In these experiments perfusion with Locke's solution was carried on for at least 20 minutes to remove all the serum. The same procedure and technique was employed for the heart of the immunised rabbit.

In the following Table III the heart of the immunised rabbit is compared with the hearts of unimmunised rabbits in respect to their susceptibility to the toxic action of the venom.

The heart of the immunised rabbit was first perfused with a concentration of venom of 1 in 400,000 for 45 minutes. In this time only a slight slowing and rise in tone was produced. The concentration of venom perfused was then doubled and arrest in systole was produced in 20 minutes after turning on the new solution.

When these effects are compared with the effects of the venom on the heart of unimmunised rabbits, it is clear that as the result of immunisation, the heart becomes much less sensitive to the action of the venom, quite apart from any antitoxic action of the serum, because in these experiments the serum is previously washed out by perfusion. A concentration of 1 in 400,000 has less effect on the excised heart of an immunised rabbit than has a solution of 1 in 800,000 on the excised heart of an unimmunised rabbit Figs. 5 and 6 show this difference in action. In fig. 5 is shown the perfusion of a normal heart with 1 in 800,000, and in fig. 6 the perfusion of the heart of the immunised animal with 1 in 400,000. It is clear that, though in the latter case the concentration perfused is doubled, the toxic effect produced is far less.

In short, the heart of a rabbit immunised to 10 M.L.D. was able to withstand with only slight changes in its action, twice the concentration of venom that sufficed to arrest the heart of an unimmunised animal.

Further, when the immunised heart was subsequently perfused with a concentration of 1 in 200,000, it was only arrested in systole in 20 minutes, corresponding to the time of arrest produced by 1 in 600,000 in the normal heart, in spite of the fact that the former had been previously perfused for 45 minutes with 1 in 400,000.

Though these experiments are insufficient to enable an exact numerical ratio to the quantitative effects of the venom upon the immunised and non-immunised heart to be given, it is justifiable to state that in a rabbit immunised to 10 M.L.D., the heart is at least three times less sensitive to the action of the venom than is the heart of an unimmunised rabbit.

Table III.—Comparison of the Action of Cobra Venom on the Normal and Immunised Heart.

Strength.	Normal rabbit.	Immunised rabbit.
1 in 200,000	Systolic arrest in 13 minutes	Systolic arrest in 20 minutes.
„ 400,000	„ „ 15 „	Very slight effects in 45 minutes.
„ 600,000	„ „ 20 „	—
„ 800,000	„ „ 34 „	—

(b) *Coronary Flow.*

As has been stated in a preceding section (p. 90), when the normal rabbit's heart is perfused with cobra venom, the rate of flow through the coronary vessels is diminished from the first. When the heart of the immunised rabbit was perfused with 1 in 400,000, there occurred a preliminary increase of flow lasting 7 minutes and amounting at its

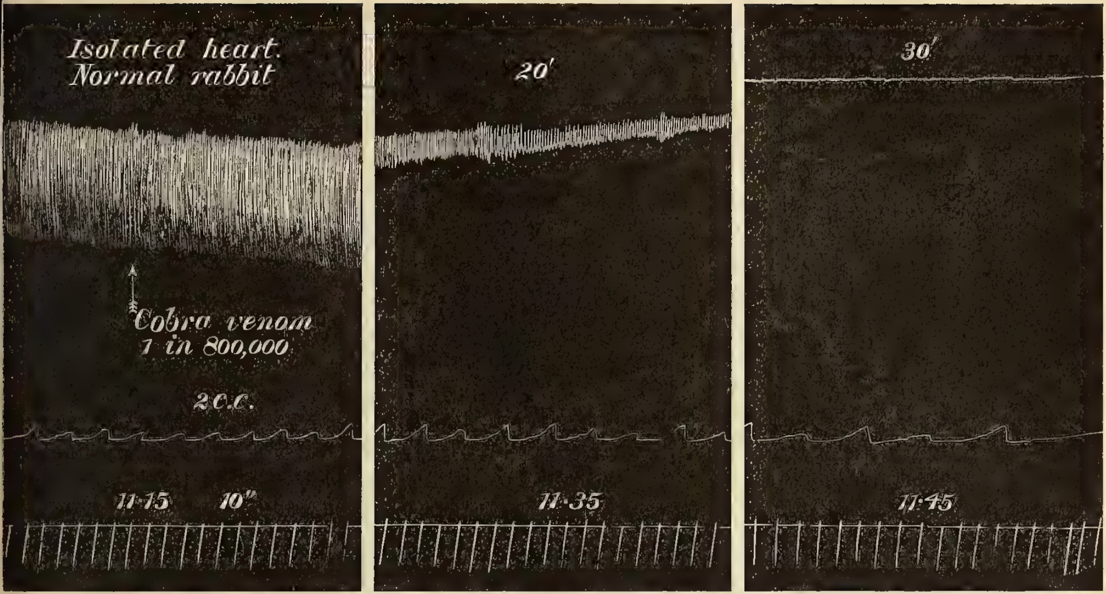

Fig. 5.—Isolated heart of normal rabbit perfused with cobra venom, 1 in 800,000.

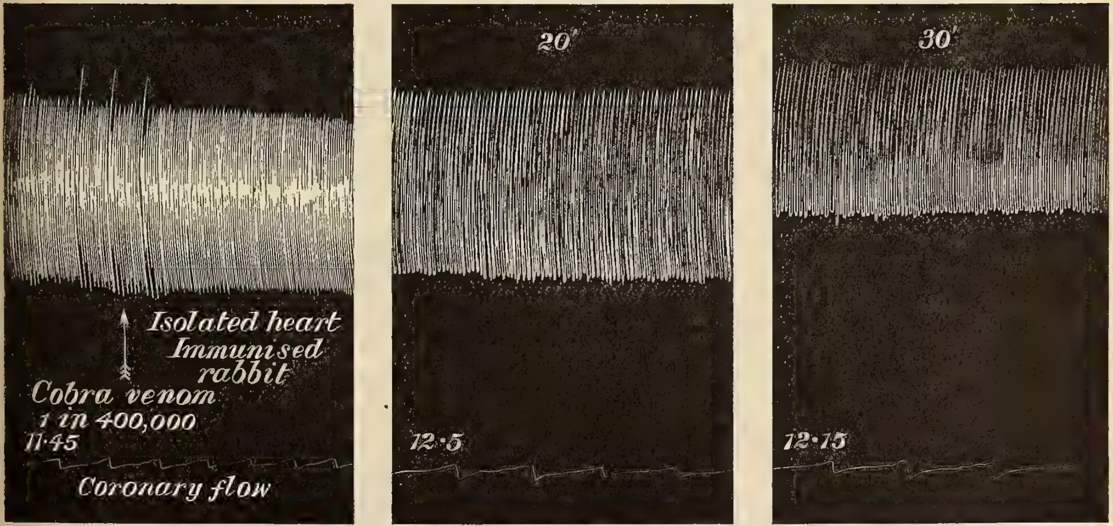

Fig. 6.—Isolated heart of immunised rabbit perfused with cobra venom, 1 in 400,000. Showing that the toxic effect on the heart of the normal rabbit is far greater than that produced, by twice as high a concentration, on the heart of the immunised rabbit.

maximum to a 50 per cent. increase 3 minutes after the venom perfusion began. It would be unwise to lay stress upon a single experiment of this kind, but it is none the less suggestive that the preliminary increase of coronary flow occurs in the cat's heart, which shows a relative natural immunity, as if the type of effect in the acquired immunity was approximating to that of congenital immunity.

(c) *Intestine.*

In order to get rid of effects due to the presence of antitoxin, it was necessary to wash out the serum from the gut previously to trying the action of the venom. For these experiments, therefore, the gut was washed out *in situ* by perfusion with warm Locke's solution by means of a cannula tied into the aorta, and connected with an elevated reservoir containing the perfusing solution. This was done both in the case of the normal and immunised animal, in order that the intestine should go through the same manipulations in each case. Segments of the gut were afterwards cut out and used for experiment in the ordinary way, and a large number of experiments could in this case be done with the gut of one animal. It was found in the case of the intestine as in the case of the heart that the muscle of the immunised animal, free from serum, was less sensitive to the toxic action of the venom than that of the normal animal. In figs. 7 and 8 is shown a comparison of the effect of 1 in 10,000 upon the normal gut with that of 1 in 5,000 upon the gut of the immunised animal. Though in the latter case the concentration was twice as great, the toxic effect is conspicuously less.

It follows from these experiments that, when an animal is immunised to cobra venom, the tissues of the gut acquire an increased resistance to the toxic action of the venom, apart from the protective action of the antitoxin serum.

(d) *Red Blood Corpuscles.*

The method of experiment and technique were the same as in the experiments previously described (p. 94).

The venom solution was again divided and used for both experiments, so that the corpuscles of the normal rabbit and of the immunised rabbit were subjected to exactly the same concentration of venom. The corpuscles were thrice washed, and the saline solution used was 0·85 per cent. sodium chloride in distilled water.

In 3½ hours the following changes had taken place:—(*a*) Normal corpuscles. Complete hæmolysis by venom in concentrations down to 0·01 per cent., partial hæmolysis with 0·005 per cent., and no hæmolysis with less concentrations. (*b*) Corpuscles of immunised animals. Complete hæmolysis by venom in concentrations down to 0·0033 per cent., partial hæmolysis with 0·002 per cent., and no hæmolysis with less concentrations.

In other words, the corpuscles of the immunised animal are more easily

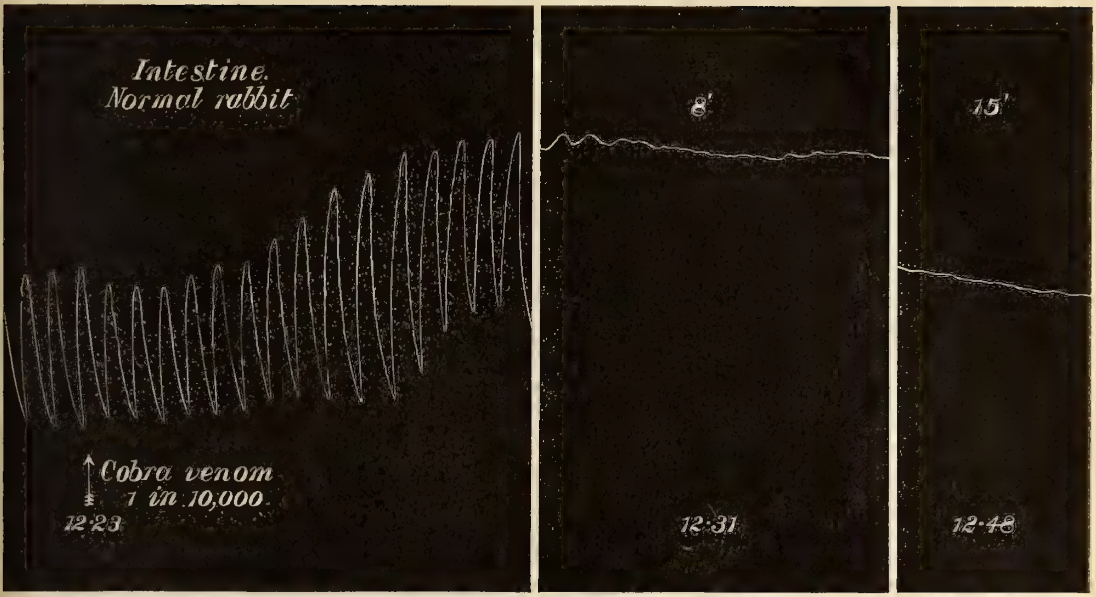

Fig. 7.—Isolated intestine of the normal rabbit.

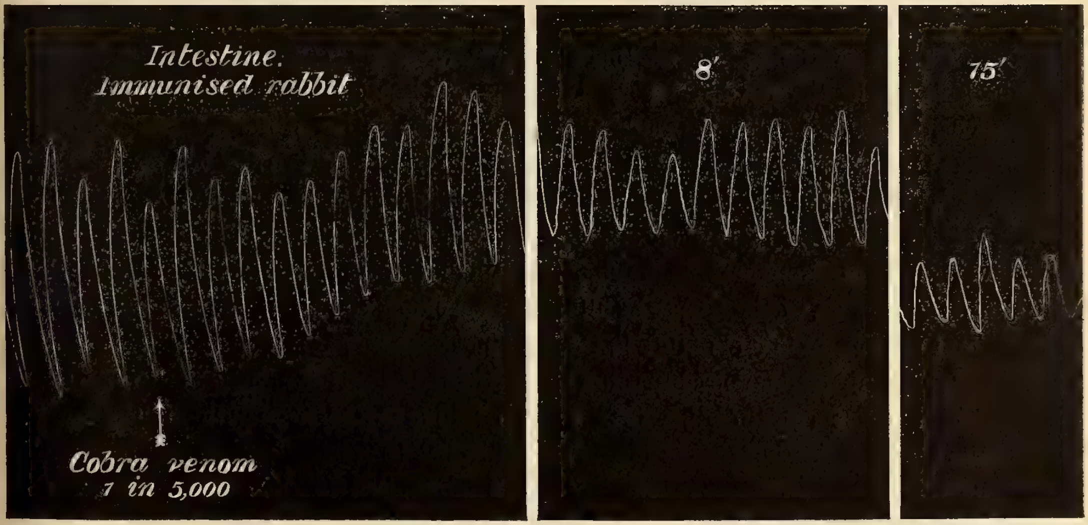

Fig. 8.—Isolated intestine of immunised rabbit. Showing that the toxic effect of 1 in 5,000 on the latter is less than that of 10,000 on the former.

hæmolysed than the corpuscles of the unimmunised animal. In the case of the rabbit immunised to 10 M.L.D., the corpuscles were hæmolysed by about one-third of the hæmolytic dose for the normal animal.

These results confirm what was found for the agglutination of red cells of immunised and non-immunised animals by ricin. The result seems to be one of considerable importance, because it shows that whereas the heart and intestine become less susceptible to cobra venom in the process of immunisation the red blood corpuscles actually become more sensitive. The most probable reason for this difference is the one suggested in the introduction to this paper, namely, that the red cells, having lost their nuclei, and being no longer living cells in the ordinary sense of the word, cannot respond to the action of repeated doses of the venom in the same way as nucleated cells.

Summary and Conclusions.

(a) *Natural Immunity.*—The minimum lethal dose of cobra venom for the cat is twenty times that for the rabbit (by subcutaneous injection per kilogramme). When the excised hearts of rabbits and cats are artificially perfused with Locke's solution so as to remove the serum, it is found that it requires at least four times as strong a solution of venom to arrest the cat's heart as is required to arrest the rabbit's heart. Similarly the isolated intestine of the cat can withstand the toxic action of higher concentration of venom than can the isolated intestine of the rabbit. The natural immunity of the cat to cobra venom is therefore, in part at least, due to a cellular immunity of the tissues of this animal. No such cellular immunity is displayed by the red-blood corpuscles, those of the cat being actually more sensitive than those of the rabbit to the hæmolytic action of the venom.

(b) *Acquired Immunity.*—When a rabbit is immunised to cobra venom, the isolated heart and intestine, perfused with Locke's solution so as to remove the serum, withstand higher concentrations of venom than the heart or intestine of a normal unimmunised rabbit. In the process of acquired immunity, therefore, some of the tissues at all events develop a cellular immunity, apart from the antitoxin circulating in the serum.

In the rabbit immunised to cobra venom the red blood corpuscles, freed from serum, become, on the other hand, more sensitive to the hæmolytic action of the venom. At the stage of immunity which has been examined, they display no cellular immunity. When a rabbit is immunised to ricin, the red cells also become more sensitive to the agglutinating action of this toxin.

It is clear, therefore, that neither in natural nor in acquired immunity can the red blood corpuscles be taken as a reliable index of cellular immunity. This difference between them and the other tissues examined is, it is suggested, due to the fact that the structure and life history of the red blood corpuscles is different from that of the other cells of the body.

While other results may occur with other toxins, and possibly even with the same toxins at different stages of immunity, the fact that a cellular immunity of such tissues as the muscle of the heart and intestine can be produced is regarded as proved.

The extent to which different tissues acquire and retain a cellular immunity, beyond and independent of the presence of antitoxin in the serum, and the universality or otherwise of the occurrence of cellular immunity with different toxins requires further investigation.

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The Effect of Thyroid-feeding and of Thyro-parathyroidectomy upon the Pituitrin Content of the Posterior Lobe of the Pituitary, the Cerebro-spinal Fluid, and Blood.

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The material used for this research was obtained from cats. There were three series of six animals, comprising three males and three females in each group. The animals were healthy adults, of as near a weight and age as possible. One set of six animals was fed with large quantities of fresh ox thyroid for from two to three weeks in addition to their ordinary diet. In the second set thyro-parathyroidectomy was performed on each animal. The cats of the third group were utilised as normal controls. The feeding with thyroid of the first group was begun some time before the operations were done upon the second group. All the animals operated upon showed typical symptoms, and were killed from three to six days after operation. The cerebro-spinal fluid was collected from the fourth ventricle of each cat under anæthesia, the animal then bled, and the blood defibrinated. The pituitary body was dissected out and the posterior lobe separated and dried on a watch-glass rapidly at 37° C. The cerebro-spinal fluid was also evaporated and dried at the same temperature. Similar procedures were adopted in the thyroid-fed animals and in the controls.

In this way there were collected three materials—dried posterior lobe of pituitary, dried cerebro-spinal fluid, and defibrinated blood—from three groups of animals—normal, thyroid-fed, and thyro-parathyroidectomised cats. The materials were then tested physiologically—by Dale's method upon the uterus of the virgin rat, by Elliott's method upon the blood pressure of a pithed cat, and by their action upon the blood pressure, kidney volume, and secretion of an anæsthetised cat.

The Posterior Lobe of the Pituitary Body.

The amount of dried posterior lobe of the pituitary was approximately the same in each group of animals. The material of each group was ground in a mortar, and solutions in Ringer's fluid made up of corresponding amounts in each case. A 0·001 per cent. extract of the dried gland was finally adopted as the standard strength for testing. This dilution of posterior lobe acts powerfully upon the rat's uterus.

No appreciable difference is obtained between the action of the material from the three groups of animals. Immersion of the uterus in each solution was limited to fifteen seconds, and both height and duration of contraction are the same in the normal, thyroid-fed, and parathyroidectomised material.

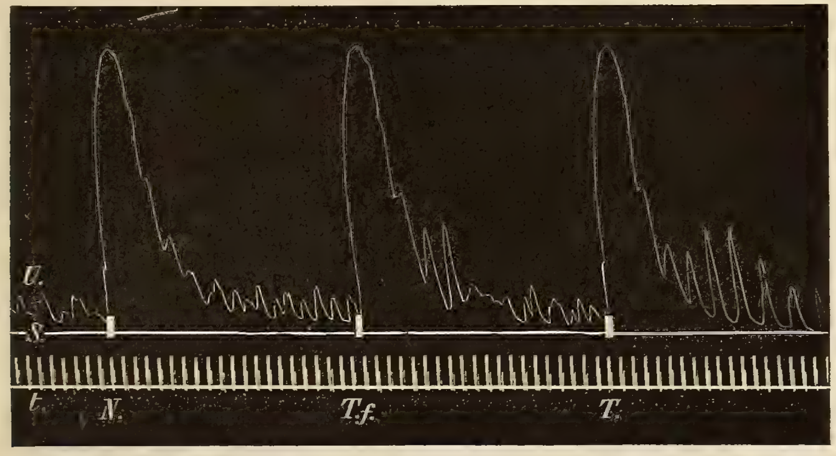

FIG. 1.—Effect upon the isolated uterus of the virgin rat of immersion in 0·001 per cent. extracts of dried pars nervosa of the pituitary body.

N., extract derived from normal cats ; *T.f.*, extract from thyroid-fed cats ; *T.*, extract from thyro-parathyroidectomised cats. The upper tracing : *U* represents the uterus ; *S*, the signal ; *t.*, the time in 30-second intervals.

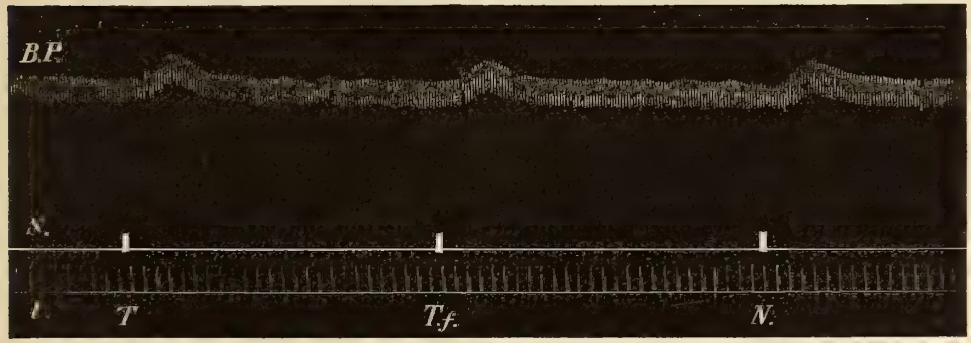

FIG. 2.—Effect upon the blood pressure of a pithed cat of the intravenous injection of 2 c.c. of a 0·001 per cent. extract of dried pars nervosa derived from

T., the pituitaries of thyro-parathyroidectomised cats ; *T.f.*, the pituitaries of thyroid-fed cats ; *N.*, the pituitaries of normal cats. *B.P.* is the carotid blood pressure ; *S.*, the signal and zero line of blood pressure ; *t.*, time in 10-second intervals.

When tested by Elliott's method upon the low blood pressure of a pithed cat, doses of 2 c.c. injected intravenously cause a small transient rise of blood pressure, but again no appreciable difference appears in the action of the three materials. The experiments were repeated and injections made in different orders, but no change in the results occurred.

It has been shown that the rat's uterus responds uniformly to the same extract of pituitary, and that the extent of the contraction varies directly with the strength of the extract employed*. The test is not sensitive to small differences, but a reduction in the strength of the solution of 50 per cent. yields an appreciable diminution in the extent of the uterine contraction. The blood pressure test in the pithed cat, which Elliott† showed to be very sensitive to minute amounts of adrenalin, is also a good method for revealing small differences in the strengths of weak solutions of pituitrin. With such minute doses, the action of pituitrin upon the circulation can be frequently repeated, and the depressor effect which usually results from a second injection, does not occur unless the first dose is a comparatively large one.

It is inferred, therefore, that there are no gross changes in the amount of pituitrin present in the posterior lobe of the pituitary body produced either by thyroid-feeding or by thyro-parathyroidectomy.

Cerebro-spinal Fluid.

The cerebro-spinal fluid from each series of animals yielded sufficient dried material to be made into three separate solutions of 1 per cent. each in Ringer's fluid. The extracts were tested upon the rat's uterus, and upon the blood pressure, kidney volume, and urinary secretion of an anæsthetised cat.

The extracts are practically inactive. A slight increase in the uterine contractions was observed after immersion of the uterus in the cerebro-spinal fluid of the thyro-parathyroidectomised cats, but was not a constant phenomenon. None of the extracts show any effect upon blood pressure, kidney volume, and urinary secretion other than that which is produced by a similar amount of Ringer's solution alone.

It would appear, therefore, that there is no appreciable amount of pituitrin in the cerebro-spinal fluid taken from the fourth ventricle, whether from normal, thyroid-fed, or thyro-parathyroidectomised cats. This does not exclude the possibility that pituitrin is occasionally liberated into the cerebro-spinal fluid in the third ventricle. If such takes place, one would expect rapid absorption to occur, and the probability is against the material reaching the fourth ventricle.

Carlson and Martin‡ were unable to obtain any pressor effect from the cerebro-spinal fluid of normal dogs. Cushing and Goetsch,§ on the other hand, found that in certain pathological conditions human cerebro-spinal fluid has the same action upon the blood pressure, kidney volume, and urinary secretion as extracts of the posterior lobe of the pituitary body.

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Defibrinated Blood.

The defibrinated blood shows little action upon the rat's uterus, and no appreciable difference is seen whether the blood be taken from the normal thyroid-fed, or thyro-parathyroidectomised animals.

When tested upon the blood pressure of an anæsthetised cat differences are at once revealed. The blood from the normal animals causes a distinct fall of arterial blood pressure accompanied by contraction of the kidneys and a slowing of urinary secretion. This is succeeded by a return to normal of the blood pressure, slight dilatation of the kidney and increased urinary secretion.

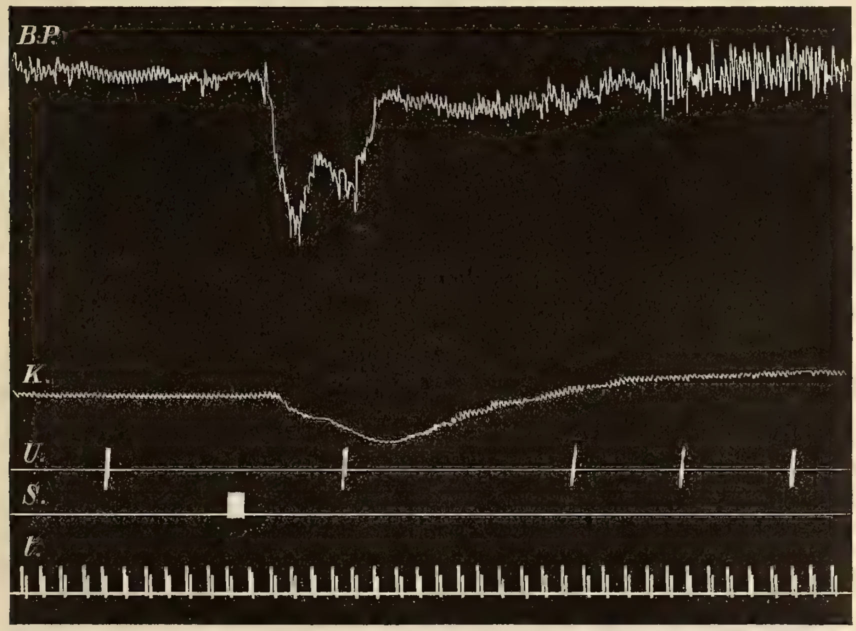

Fig. 3.—Effect upon blood pressure, kidney volume and urinary secretion (cat) of the intravenous injection of 3 c.c. of defibrinated blood of normal cats. *B.P.*, carotid artery blood pressure; *K.*, kidney volume; *U.*, urine drops; *S.*, signal; *t.*, time in 10-second intervals.

The blood from the thyroid-fed cats has a similar but more pronounced action, the blood pressure falls lower, indicating the presence in the blood of the thyroid-fed animal of more depressor material than is present in the normal animal.

The blood from the thyro-parathyroidectomised cats, on the other hand, shows a slight depressor action followed by a distinct pressor effect which lasts for some time. The kidney shows a transient contraction, and the flow of urine is distinctly diminished.

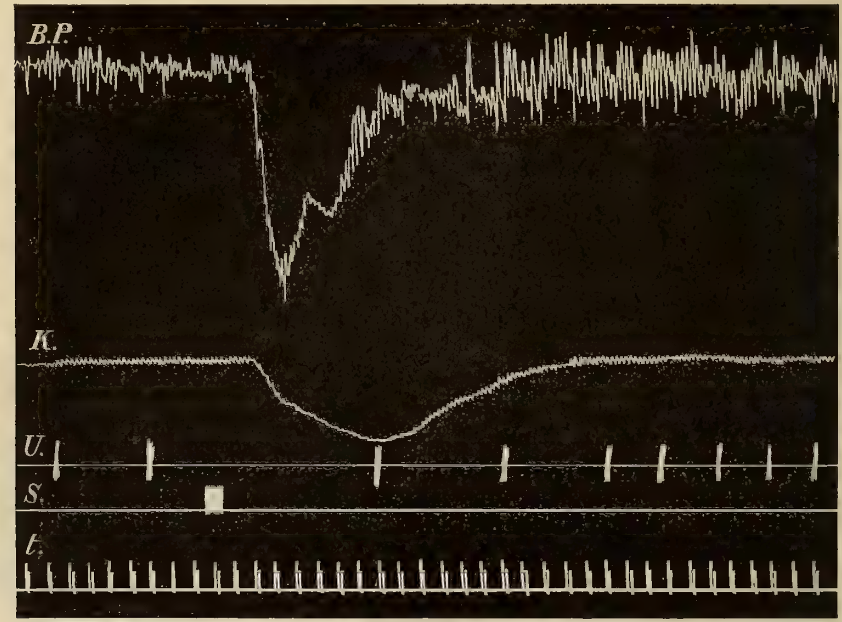

Fig. 4.—Effect upon blood pressure, kidney volume, and urinary secretion (cat) of the intravenous injection of 3 c.c. defibrinated blood of thyroid-fed cats.

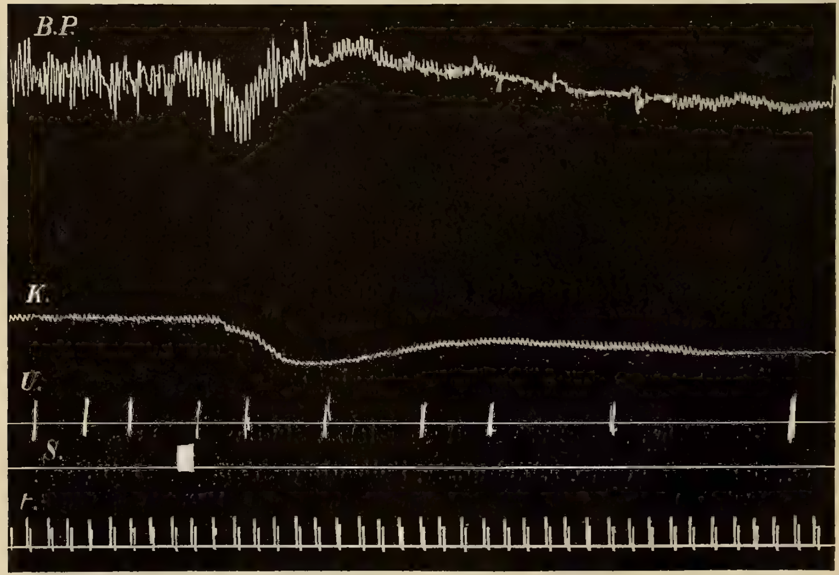

Fig. 5.—Effect upon blood pressure, kidney volume, and urinary secretion (cat) of the intravenous injection of 3 c.c. defibrinated blood of thyro-parathyroidectomised cats.

As the thyro-parathyroidectomised animals showed symptoms of tetany before death it is probable that the pressor action of their blood is due to the presence in it of guanidin, or allied bodies, as described by Noël Paton* and his co-workers.

Summary.

1. Neither thyroid feeding nor thyro-parathyroidectomy affects the pituitrin load of the posterior lobe of the pituitary body as tested by the action of extracts upon the contraction of the rat's uterus and the blood pressure of the pithed cat.
2. There is no evidence of the presence of pituitrin in the cerebrospinal fluid of the fourth ventricle in normal, thyroid-fed and thyro-parathyroidectomised cats.
3. The defibrinated blood of normal, thyroid-fed and thyro-parathyroidectomised cats shows no appreciable differences in its action upon the rat's uterus. The blood of the thyroid-fed cat has a greater depressor action upon the circulation than has that of the normal animal. The blood of the thyro-parathyroidectomised cat exercises a pressor effect upon the circulation, accompanied by a contraction of the kidney and a diminution in the secretion of urine.

The expenses of the research have been met by a grant from the Research Fund of the Carnegie Trust for the Universities of Scotland.

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* Noël Paton, 'Quart. Journ. Exper. Physiol.,' vol. 10, p. 203 (1917).

On the Myogram of the Flexor-reflex evoked by a Single Break-shock.

By K. Sassa and C. S. Sherrington, Pres. R.S.

(Received December 20, 1920.)

(From the Physiology Laboratory, Oxford.)

Our object has been to compare the reflex contraction of a muscle when evoked by a single induction shock with the contraction evoked from the same muscle when a similar stimulus is applied directly to the muscle's motor nerve. The experiments extend some observations previously reported in these Proceedings (2) and in the 'Journ. of Physiology' (6). The literature of the subject was given in those papers and for it reference may be had to them.

Method.

The reflex preparation employed has been the spinal cat, the muscle being tibialis anticus. Either before or after the spinal cord had been transected the animal was decerebrated. The spinal transection and the decerebration were performed under deep anæsthesia. The spinal transection was in some cases made three to ten days prior to the decerebration. The seat of spinal transection was usually at the posterior end of the thoracic region, but sometimes at the anterior end of the cervical region. All the hind-limb muscles except tibialis anticus were immobilised by nerve section, or in the case of extensores, digitorum and peronei, tenotomy. The limb was fixed by steel drills in tibia and femur, the drills being clamped to unyielding uprights on the experimental table. The tendon of tibialis anticus was attached by a short length of waxed fishing line to an isometric myograph of the torsion-wire pattern. The vibration period of this recorder was somewhat less than 0·01″. The afferent nerves of the limb were severed and those used for stimulation were popliteal in the ham, musculo-cutaneous on the dorsum of the foot, and internal saphenous, femoralis, and external cutaneous below the groin.

The break induced current used as stimulus was obtained by the automatic opening of a contact in the primary circuit. The striker opening the contact was attached to a spindle carrying the recording surface, the moment of delivery of the stimulus being thus registrable on that surface. The spindle was horizontal and rested on bearings similar to those of an Atwood machine; it was operated by the fall of a weight. After the reflexes and their latencies had been registered the nerve of the muscle was severered and its distal stump stimulated for obtaining the motor twitch. For stimulation of the afferent nerve the break-shock kathode was placed nearer to the spinal cord, for the efferent nerve it was placed nearer to the muscle.

Results.

It will be convenient to refer to the contraction evoked by the break-shock applied to motor nerve as the neuromyal twitch.

(1) *Duration of Contraction.*—The reflex myogram was almost always of longer duration than the maximal twitch myogram. This was so even when the height of the reflex myogram was considerably less than that of the twitch (2). Often the excess in duration of the reflex contraction over the maximal twitch was great; the reflex contraction lasted sometimes even four or five times as long as the twitch contraction. The reflex contractions evoked from musculo-cutaneous nerve on the dorsum of the foot (skin) and from internal saphenous were usually of longer duration than that evoked from the popliteal, although their contraction height was less. In spite of general similarity of form the reflex from the several nerves was found to present to some extent features characteristic for the nerves severally. The prolongation of the reflex contraction lay chiefly in the prolonged subsidence of the contraction after the crest of the myogram had been reached (fig. 1).

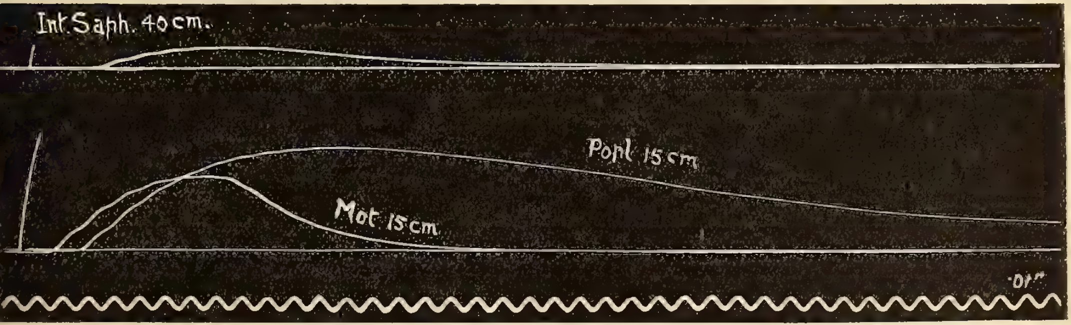

Fig. 1.—Break-shock reflexes (spinal preparation) of tibialis anticus; upper line, internal saphaneous nerve stimulated with secondary coil at 40 cm. from primary; lower line, popliteal nerve stimulated, coil at 15 cm., and, for comparison, a maximal twitch contraction given by stimulating motor nerve (peroneal) with coil at 15 cm. (break-shock). Time in 0·01″.

(2) *Crest-height.*—With the isometric myograph the height of the myogram is an index of the degree of tension developed by the contraction. In the reflex contractions the tension crest was reached relatively later after onset of the contraction than in the neuromyal twitch. In most of our preparations

a reflex crest-height could be obtained exceeding that of the maximal neuromyal twitch (2) (6), and with the secondary coil no nearer to the primary for the reflex than for the twitch. To obtain this excess of contraction height above that of maximal twitch the stimulus had always to be much above threshold (4) (7). Under successive increase of the induction-shock stimulus the successive increase of the reflex contraction (flexor) runs a course different from that of the neuromyal twitch. In the latter, as is well known, the maximal twitch is reached soon and then remains constant throughout a long range of further increment of stimulus (maximal stimuli). In the reflex the contraction goes on increasing through a far longer range of stimulus increments; it is in the upper part of this series that the reflex contraction exceeds the maximal twitch. The result was obtained from any one of the afferent nerves used for the reflex, but most readily from the largest of them, the popliteal in the ham. That the superiority of the height of the contraction excited with the electrodes on the afferent nerve was not due to escape of the current to the motor nerve or muscle, thus adding a twitch contraction to the reflex, was shown by the latent interval of the response remaining of characteristic reflex length (fig. 2).

(3) *Steepness of Ascent.*—The ascent of the myogram of the reflex contraction is not unfrequently for part of its course more steep than is any part of the ascent of the maximal twitch. This tends to occur especially when the crest-height of the reflex is greater than that of the maximal twitch.

(4) *Abruptness of Contraction.*—In the myogram of the maximal neuromyal twitch, the vibrations of the recording lever are more evident than in the myogram of the reflex contraction, an indication that the first onset of the pull on the lever is more abrupt in the twitch contraction than in the reflex contraction. Forbes and Gregg (3) pointed out that, in their galvanometric records of the action-current of the motor nerve, the rise of the current was of sharper development with motor-nerve stimulation than with reflex stimulation. In some experiments we transected the cord several days before the myographic examination. We thought thus to favour the freedom of the flexor reflex by allowing time for subsidence of spinal shock. We found, however, that when a week or more—our longest period was fourteen days—is allowed to elapse after the spinal transection, change ensues in the nerve-muscle preparation itself. The maximal twitch of tibialis anticus, in response to a break-shock applied to its motor nerve, is then feeble and sluggish, of low crest-height, and of abnormally prolonged decline. The duration of the twitch may then be twice or thrice the normal. And in this condition, the reflex contractions, as elicited by a single-shock from afferent nerve or skin, are poor and sluggish, their crest-height

seemingly even lower than can be accounted for wholly by the impaired state of the peripheral nerve-muscle apparatus itself. The wasting of the limb-musculature is also very obvious. The condition was clearly less favourable for the observations we had in view than that obtaining in the earlier periods after the transection.

Discussion of Results.—1. In the assemblage of striped fibres composing a muscle, the modes of summation of their contraction are of course two. One of these is that exemplified in the twitch contraction. There, in each of some or all of the component fibres of the muscle, a single wave of contraction is generated; the occurrence of this wave is practically simultaneous in all the contracting fibres. These individual mechanical tensions of the several component fibres of the muscle sum additively; the tension developed by the muscle, as a whole, gives the resultant tension of this single-wave contraction. The upper limit of the tension-effect of this summation of a single-wave contraction, coincident in time in the individual fibres of the muscle, can be found for a given muscle from the isometric record of its maximal twitch. Where the given muscle develops a tension superior to that developed in its maximal twitch, there must be at play, above and beyond summation of the above kind, some amount of that other well-known mode of summation due to fusion of successive contraction-waves ensuing within the self-same individual fibres of the muscle. We may for brevity distinguish these two kinds of summation as "fibre summation" and "wave summation" respectively.

Applying this to the above-mentioned result, namely, to the excess of crest-height observed in the reflex contraction as contrasted with the maximal twitch when the former is excited by a break-shock of considerably above threshold value, yet not greater than that used for the compared maximal twitch, we infer that there is in the reflex contraction a factor of wave-summation in addition to the fibre-summation. That is, a single break-shock stimulus, which evokes in the neuromyal preparation a single wave contraction, evokes when applied to the afferent nerve a succession of contraction waves, at least in some of the fibres of the muscle.

2. A possible reason for this might be that a proprioceptive reflex, initiated by the contracting muscle itself, appended itself to the reflex contraction evoked by the extrinsic afferent nerve. In the spinal preparation, a proprioceptive reflex is easily elicited from tibialis anticus (Asayama) (1), *e.g.*, by a brief pluck upon the severed tendon of the muscle. We therefore proceeded to observations in which the afferent nerve-fibres from the muscle had been severed from their connections with the spinal cord. The afferent nerve-fibres from tibialis anticus, and, indeed, from all the pre-tibial and post-

tibial crural and pedal muscles pass to the spinal cord *viâ* the ipsilateral dorsal roots of the 6th, 7th, 8th, and sometimes 9th post-thoracic spinal nerves (5). Severance of these roots leaves still open for reflex play upon the muscle several important afferent nerves of the limb, namely, internal saphenous, femoralis, and external cutaneous. The two former will have lost some of their afferent fibres, but will yet retain, as experience showed, enough to evoke extensive reflexes. The motor nerve-fibres to tibialis are, of course, untouched by the severance.

Our procedure has been to cut, with full aseptic precautions and under deep anæsthesia, the dorsal roots of the 6th, 7th, 8th, and 9th post-thoracic nerves of one side, then a few days later to sever the cord aseptically at the 12th thoracic segment, and finally a few days later to decerebrate and proceed in the same manner as before to the myographic observations of the reflex and neuromyal contractions. As afferent nerves employed for evoking the reflexes, our choice was, of course, limited to internal saphenous, femoralis, and external cutaneous. By femoralis nerve is meant the whole anterior crural trunk except internal saphenous. It includes, therefore, the afferent nerve fibres from the quadriceps, extensor muscles, etc. Our observations found that the break-shock reflexes elicited showed still (figs. 2 and 3) the character of being, when the break-shock was of considerably above threshold value, both longer and of greater crest-height than the maximal twitch elicited by a similar stimulus applied to motor nerve itself. That the afferent arc of tibialis anticus, etc., had been broken was guaranteed in each experiment in several ways. For instance, by trial to obtain the proprioceptive reflex of the muscle, this reflex proving to be inelicitable in each case, though easily provocable in the contralateral limb. Also by *post-mortem* examination of the spinal roots in the vertebral canal, which showed that the roots severed had been those required: in two cases the posterior half of the 5th root had been cut as well. Also by inability to provoke by any means any reflexes from the peroneal or popliteal nerves. The knee-jerk was also entirely absent in three cases and extremely slight in two others. But as regards the crest-height and duration of the break-shock reflex elicitable in tibialis anticus, these remained often, when the stimulus was considerably above threshold strength, superior to those of the maximal motor twitch-contraction. We obtained, therefore, no support for the supposition that that superiority was founded on combination of the break-shock reflex evoked from the extrinsic afferent with a proprioceptive reflex initiated reflexly in the contracting muscle itself.

3. That the reflex contraction develops more than one single contraction-wave (per fibre) is evidenced not only by the excess of crest-height above

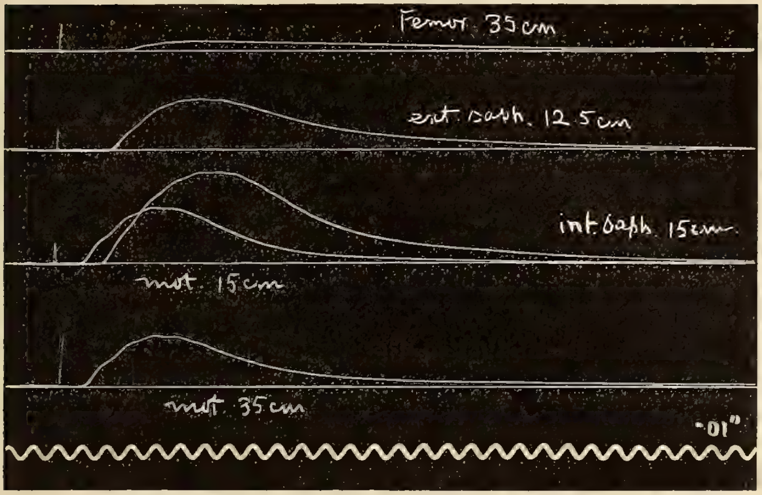

Fig. 2.—De-afferented tibialis anticus (spinal preparation); top line, reflex response to break-shock, coil at 35 cm. applied to central end of femoral nerve; second line from top, reflex to break-shock, coil at 12·5 cm., applied to external cutan-nerve; third line from top, reflex to break-shock, coil at 15 cm., applied to central end of internal saphenous nerve; and maximal twitch from break-shock, coil at 15 cm. applied to peroneal nerve; bottom line shows that twitch is already maximal under break-shock with coil at 34 cm. Time in 0·01″.

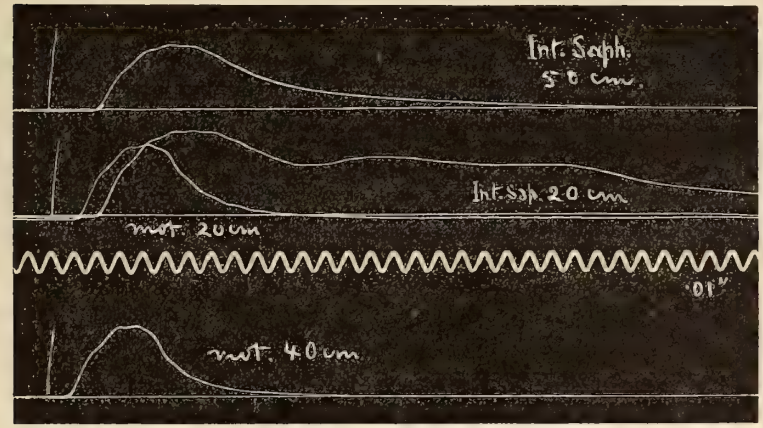

Fig. 3.—De-afferented tibialis anticus (spinal preparation); top line, reflex to break-shock, coil at 50 cm. applied to internal saphenous nerve; second line, similar but with coil at 20 cm., a maximal twitch is given for comparison with coil at 20 cm. and stimulus of motor (peroneal) nerves; bottom line, a twitch with motor nerve stimulation (break-shock) at 40 cm., showing that twitch is already maximal. Time in 0·01″.

that of the maximal twitch, but also by several other features of the myogram. (1) The excess, often very great, of the duration of the reflex contraction. It may persist for several times as long as does the maximal twitch. (2) The incidence of the crest of the contraction, also when that crest-height exceeds that of the maximal twitch, falls later after onset of contraction in the reflex than in the twitch. (3) In the reflex contraction there not infrequently ensues after the main crest a second, or even a third contraction-crest, breaking the line of subsidence of the reflex, and of period far too slow to be referable to the vibration-period of the recording apparatus. (4) The ascent of the reflex myogram is sometimes in part of its course more steep than is any part of that of the maximal twitch; this is difficult to understand except as due to summation of repetitive contraction-waves in individual muscle-fibres. It sometimes occurs as early as the 15th σ in the course of the contraction.

4. Reflex contraction of tibialis anticus is readily elicitable by a single induction shock applied to the skin of the limb, *e.g.*, to the skin of the foot. We have compared the isometric myograms of this reflex contraction (fig. 4) with the maximal twitch evoked in the same preparation from the motor nerves.

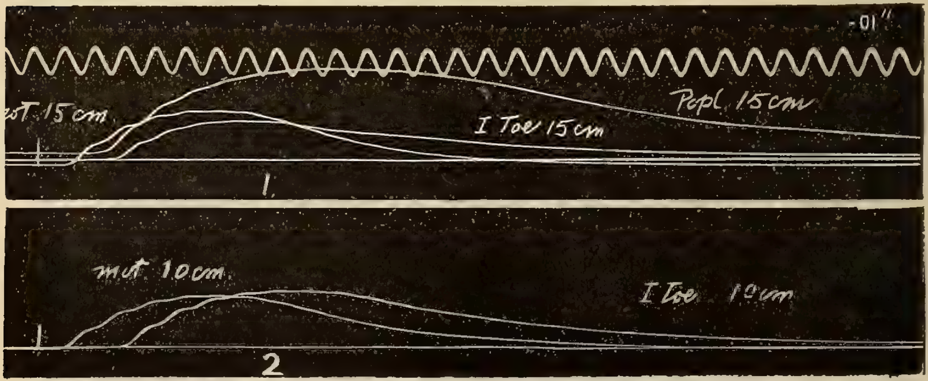

Fig. 4.—Reflex contractions of tibialis anticus (spinal preparation) in response to single break-shock applied to skin (unipolar); top line, three myograms given by break-shock, coil at 15 cm., applied respectively to skin of first digit, to central end of popliteal nerve, and to distal end of peroneal nerve (maximal twitch); bottom line, reflex from skin of first toe, coil at 10 cm. compared with motor nerve stimulation also at 10 cm., *i.e.*, maximal twitch. Time in 0·01″.

To evoke the reflex the break-shock was applied as follows: A broad copper plate was fastened to the sole of one forefoot, a layer of cotton-wool soaked in strong salt solution intervening between the skin and the plate. For the stigmatic electrode the point of a small entomological gilt pin, attached to very thin wire, was inserted a millimetre or less into the skin of the dorsum of one of the hind toes, and the wire brought to one binding screw of the short circuit key of the secondary circuit, the wire from the copper plate being brought to the other binding screw of the key. The stigmatic electrode was made the kathode for the break-shock. The registration, etc., was as in other experiments.

The myograph records showed that, with break-shocks considerably above threshold value, the reflex contraction was often of greater crest-height, as well as of longer duration, than was the maximal twitch contraction evoked from the motor nerve. The myogram of the break-shock reflex elicited from the skin resembled that of the break-shock reflex evoked from the bared skin nerve, *e.g.*, musculocutaneous nerve, except that the skin reflex tended to be somewhat less prolonged.

5. In the spinal preparation, reflex contraction of tibialis anticus and other flexor muscles of the limb is obtained from the ipsilateral afferent limb nerves more readily than in the decerebrate preparations (7), and the reflex contraction is more ample. In some of our experiments, we have taken myograms of the tibialis anticus break-shock reflex in the decerebrate condition, and then in the spinal condition, the spinal transection being made either in the posterior thoracic region or in the anterior cervical. Both with the low and the high spinal transection, the difference between the decerebrate reflex and the spinal has been great (fig. 5). Very rarely has the crest-height of the decerebrate reflex equalled that of the maximal twitch evoked *viâ* motor nerve. Often it has been less than half of that even with break-shocks much higher on the inductorium scale than those sufficing for the maximal twitch. In our experience, the break-shock reflex contraction of tibialis anticus evoked from even the popliteal nerve in the decerebrate condition gives a low relatively flat myogram of rather prolonged duration, longer usually than that of the maximal twitch. The reflex provoked from the same afferent nerve, and by a similar stimulus in the spinal preparation, may have a crest-height four or five times that of the decerebrate reflex (6), (7). This greater reflex response in the spinal, as compared with the decerebrate preparation, is obvious immediately, *e.g.*, ten minutes after spinal transection. That it is, however, a release phenomenon rather than merely an irritative excitation from the fresh trauma, is indicated by its persistence, not only for many hours but for some days, to judge from the similar character of the reflexes obtainable, when the cord had been cut, as in some of our experiments, a week or more prior to the myographic examination. Also, the threshold value of stimulus (break-shock) is higher, often far higher, in the decerebrate (6), (7), than in the spinal condition (6), (7). Also, the upper

stage of the grading of reflex response to a single-shock is, with the flexor muscle, much less extensive in the decerebrate than in the spinal preparation.

6. To summarise, we find that in the spinal preparation a single break-shock, whether applied to a bared afferent nerve or to local skin, evokes when the shock is considerably above threshold value a reflex contraction which is both stronger and more prolonged than is the maximal twitch evoked from the motor nerve itself, and that this is so whether or no the muscle retains its own intrinsic proprioceptive reflex arc. This excess of the reflex contraction over the maximal twitch indicates that summation of successive contraction-waves is present in the reflex contraction. So, further, does the occurrence, not unfrequent, of a more rapid up-gradient of tension development, despite less abrupt first onset of tension development, in the reflex than in the maximal twitch. In other words repetitive discharge from the reflex centre occurs in response to a single induction shock stimulus applied to an afferent nerve or to local skin connected with that centre.

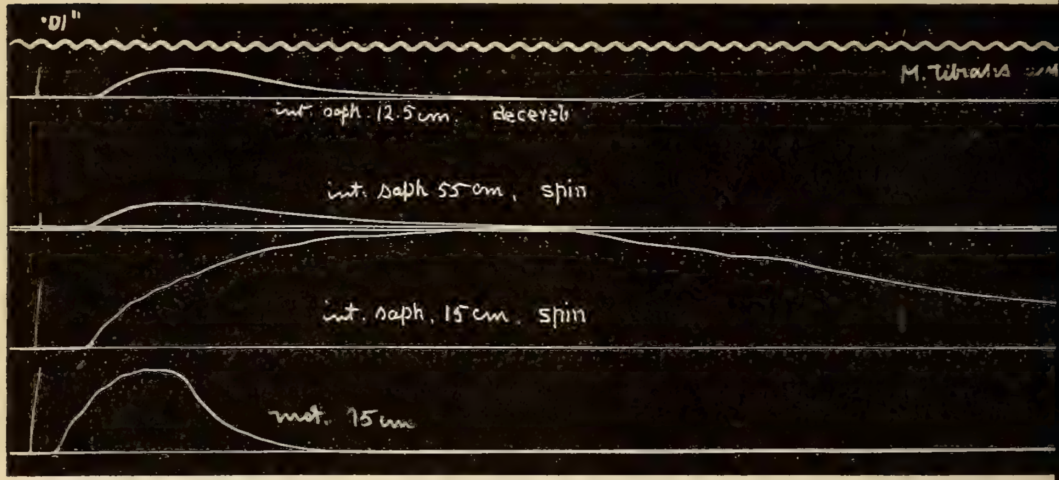

Fig. 5.—Reflex contraction of tibialis anticus in response to single break-shock stimulus of afferent nerve trunk compared in the decerebrate (top line) and spinal (second and third lines from top) conditions respectively, and with a maximal twitch from motor nerve (bottom line); the coil at 12·5 cm. in the top line, and at 15 cm. in third and bottom line; at 55 cm. in the second line from top. Time in 0·01".

It may be that the stimulus applied to the afferent nerve or to the skin sets up a local change which, before it subsides, originates there not merely one but a short series of nerve impulses. We are examining this possibility further. If it be so it is remarkable that the similar stimulus supplied to the

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Every paper must be accompanied by a summary not exceeding 300 words in length.

Authors are requested to refer to a Memorandum on Mathematical Notation published in these 'Proceedings,' 1909, Series A, vol. 82, p. 14, and to adhere to the suggestions therein contained, so far as possible.

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motor nerve excites there not a repetitive series of impulses but (per fibre) one single impulse only, evoking a twitch contraction, not in a tetanic contraction.

On the other hand, it may be that the spinal reflex centre on receipt of a single-impulse volley from the afferent nerve responds by an impulse-discharge repetitive in character. Such a result might be due to the impingement upon the centre's cells severally of numbers of terminals coming from the afferent path, so that upon any one central neurone there converged a number of excitatory impulses, some arriving later than others. Or it may be that across the central path of the reflex there is some structure, *e.g.*, a synaptic membrane, which has a property of repetitive activity in result of, or in response to, the arrival at it of even a single impulse or of a single simultaneous set of impulses.

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On "Intertraction" between Albuminous Substances and Saline Solutions.

By Sir ALMROTH E. WRIGHT, M.D., F.R.S.

(Received November 30, 1920.)

In 1906 I pointed out that hypertonic salt solutions applied to wounds, sinuses and foci of infection increased the discharge from these, supplementing the ordinary mechanical drainage by drawing out from the tissues infected and corrupted lymph.

In the war, and especially in its earlier period when every form of sepsis and gangrene was rife and almost every wound was foul, I again advocated treatment by hypertonic salt solutions. The method was then employed extensively and with good results.

Hereupon followed a detailed study of the action of hypertonic salt solutions upon the wound, and also an examination of their action *in vitro*.* It was in the course of this latter found that when a receptacle containing strong salt solution is connected up with a receptacle containing water by a siphon tube threaded with a wick or filled with water and armed (at the end which dips into the salt solution) with a tight cottonwool plug, water is slowly drawn into the salt solution—the level of this rising and that of the water falling.

A much more rapid and abundant drawing effect—in the form of a down draught of supernatant fluid into a heavier salt solution—was obtained by taking a test-tube, dividing it up into an upper and lower compartment by a plug of cottonwool soaked in a solution of white of egg possessing a specific gravity of 1026 and then filling the lower compartment (through a lateral opening) with a saline solution possessing a specific gravity of 1052, and the upper chamber (which here provides a control) with water. It was found that the egg albumen was under these conditions carried down rapidly and in large quantity into the subjacent heavier salt solution while none found its way into the superjacent water.

That experiment would seem to suggest that the forces of diffusion are at any rate in the case where albuminous substances and saline solutions are brought into conjunction, supplemented by what I should like to call "*forces of intertraction.*"

In the following that hypothesis is subjected to certain further examination.

The method of investigation adopted was to superimpose serum or other albuminous fluids directly upon heavier saline solutions, or upon occasion lighter salt solutions upon heavier albuminous fluids—adding generally to one or other fluid a trace of colouring matter (eosin) in order to render the course of events more manifest to the eye. The experiments were conducted in capillary tubes, full sized test-tubes and also in a very convenient form of diffusion cell suggested by my colleague, Dr. Alexander Fleming. The diffusion cell just referred to is made by covering a microscopic slide, or larger sheet of glass with a layer of wax of any desired thickness; cutting out a cell of any desired shape; and then bringing down upon the border of wax a companion microscopic slide or glass plate.

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* 'Proceedings Royal Institution,' March 9, 1917; and 'Lancet,' June 23, 1917.

The phenomena described below manifest themselves alike in each of the above mentioned types of receptacle. For demonstration and final experimentation the flat cell has of course obvious advantages. When in such a cell serum is allowed to run down gently from a pipette on to the surface of a heavier—*e.g.*, 6 per cent.—saline solution the following train of events occurs.

When the serum impinges upon the surface of the salt solution it indents it, and then takes up by hydrostatic resilience a position on the surface, floating there as a layer which is delimited below by a somewhat undulate outline. Then within a very few seconds—seemingly as a result of whirlpool movements sucking in the wave summits protruding downwards from the under surface of the serum—this specificially lighter fluid is drawn down into the heavier salt solution below. The appearance is then as if pseudopodia or tentacles were being let down into the depths (figs. 1 and 2). Simultaneously with this, as can be seen when we employ a coloured salt solution and an uncoloured serum, the former is carried up into the serum forming there a system of thin ascending streams arranged after the fashion of the teeth of a comb (fig. 2).

This down- and up-streaming process progresses apace and gives, as an intermediate result: a stratum of transported serum upon the floor of the cell; and a layer of transported salt solution ranged at the top of the cell superficially to the original stratum of serum. As a terminal result, we have complete interfusion, manifested to the eye by quite uniform coloration. With a diffusion cell made from microscopic slides (*i.e.*, a cell measuring 1 inch by 3 inches) this is arrived at in something like half-an-hour.

It will be seen that we have here two arresting features: the singular fashion in which the lighter and heavier fluids interpenetrate (we may perhaps speak of this as "*pseudopodial interpenetration*"), and the rapidity with which complete interfusion is achieved.

The singular point about the pseudopodial interpenetration is not so much that a lighter fluid (the serum) is carried down into a heavier one; but that this serum, instead of recoiling to the top, sinks to the bottom, like a heavier fluid, descending in definite streams to spread itself out upon the floor of the cell. Exactly the same applies to the heavier salt solution. It is not only caught up into the serum, but continues to ascend there in definite streams.

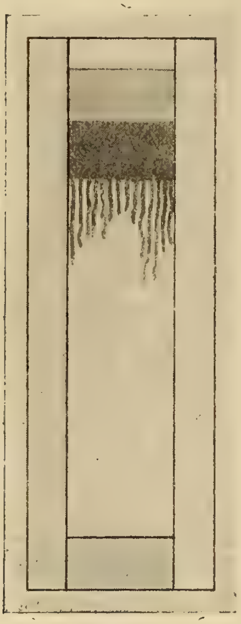

Fig. 1.

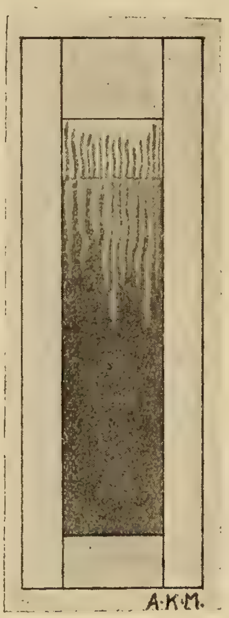

Fig. 2.

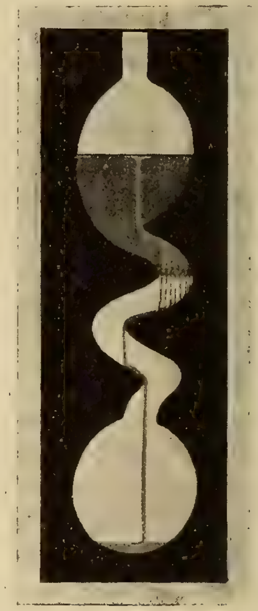

Fig. 3.

Fig. 1.—Flat diffusion cell filled in with 6 per cent. NaCl solution below. Upon this is superposed coloured serum; and again upon this, water. The specifically lighter serum is seen interpenetrating in the form of pseudopodial processes into the heavier subjacent salt solution.

Fig. 2.—Flat diffusion cell filled in below with coloured 6 per cent. NaCl solution. Upon this is superposed uncoloured serum. The subjacent coloured salt solution is interpenetrating into the supernatant coloured serum, and the uncoloured serum into the subjacent coloured salt solution.

Fig. 3.—Flat diffusion cell filled in below with uncoloured 6 per cent. NaCl solution, and above with coloured serum. The ascending rills of uncoloured salt solution here unite to form an ascending stream; and in like manner the descending streams of coloured serum unite to form a cascade.

This process of descending and ascending can be beautifully demonstrated in a specially shaped diffusion cell, which again was suggested by Dr. Fleming. The cell in question has an upper and a lower reservoir (somewhat after the fashion of a Kipp's apparatus), connected by a much narrower channel, which zigzags down after the fashion of a flattened out spiral (fig. 3). The apparatus is charged with a 6 per cent. salt solution as far as the neck of the upper chamber, and then this last is filled with coloured serum. In a cell thus charged the serum descends through the salt solution as a coloured waterfall, forming at each turn of the channel a separate cascade. At the same time, in the upper reservoir the uncoloured salt solution ascends rectilineally in a thin stream.

It may be adventured as a suggestion that the serum enters into conjunction with salt so as to become heavier, and that the saline solution, parting with its salt to the serum, becomes lighter. There is no question (when operating with unaltered serum) of any falling out of solution of the albuminous substances.

Where, as in the experiments above cited, we set out to obtain evidence of a down draught of serum and an up draught of salt solution, the salt solution employed must contain more than 4 per cent. of salt. For a 4 per cent. solution of salt has a specific gravity equivalent to that of serum (human serum).

When we want to arrange for the reverse effect—that is, for an up draught of serum into a lighter salt solution, or a down draught of this into serum—we are by necessity tied down to the employment of solutions containing less than 4 per cent. of salt. With these weaker solutions dramatic pseudopodial interpenetration and very rapid interfusion are not to be expected, and to achieve effects manifest to the eye, we may best (guiding ourselves here by the principles enunciated by Horace Brown) betake ourselves to diffusion cells of conical section. To achieve a visible up draught of serum we require a cell corresponding in shape to the section of an inverted funnel.

Into such a cell we introduce a layer of coloured serum, and then superimpose a 3 per cent. solution of salt, filling in with this the remainder of the contracting cone and also the stalk of the funnel. At the same time, for the purpose of a control, we fill in a second cell, employing here, as our recipient fluid, water instead of salt solution. It will, after the lapse of a few hours, become manifest that the serum is being caught up into the salt solution, and that it is not sensibly diffusing into the water.

The down draught of a lighter salt solution into serum can—taking here again as our guide the principles of Horace Brown—be made manifest to the eye by employing a diffusion cell of triangular shape, disposed with its apex downwards, and filling into the apex of this expanding cone uncoloured serum, and superimposing coloured 3 per cent. salt solution.

Passing on now, the following points may be briefly adverted to:—

Where serum and salt solution are brought into conjunction, the content in albuminous substances plays a very important part in the production of the phenomena described above. When we take a series of capillary tubes, place a fiducial mark upon the upper part of the stems, fill in up to that level with a 6 per cent. salt solution, seal up the distal ends of the tubes, and then with a paraffined hair fine pipette superimpose—in the one tube, upon the salt solution a coloured serum; and in the others progressive dilutions of this—the drawing effect of the salt becomes progressively less manifest, becoming almost inconspicuous when we reach a 32-fold dilution of the serum.

The concentration of the salt also influences the result. The optimum display of pseudopodial interpenetration and the most rapid interfusion would appear to be obtained when serum is superposed upon 5 per cent. to 8 per cent. sodium chloride solutions. Very concentrated solutions give less striking results, this being presumably due to the greater resistance which these heavier fluids would offer to the down draught of serum.

We have already seen in the experiment with the cottonwool plug which furnished the starting point for these experiments, that solutions of egg albumen react with salt in the same way as serum. This would seem to hold true also of albuminous substances obtained from muscle.

Solutions of commercial peptone give only an indistinct reaction.

All the commoner salts—such as sodium sulphate, potassium chloride, potassium sulphate, and magnesium sulphate—react with serum in apparently the same manner as sodium chloride. The same holds true of solutions of cane sugar, and here again very concentrated solutions give less striking results.

As in the case of diffusion proper, so here temperature exerts a dominating influence. In an experiment conducted by superimposing coloured serum upon 6 per cent. salt solution in diffusion cells made out of pairs of microscopic slides, the time occupied in the descent of the serum to the floor of the cell (a distance of about 2½ inches) was found at a temperature of 45° C. to be forty-two seconds; at 15° C., one minute fifteen seconds; and at 3° C., three minutes.

Before embarking upon any general comment, the data of certain other experiments which have a bearing upon the employment of hypertonic salt solutions in the treatment of foul septic wounds may be briefly put on record. The experiments are as follows:—

Experiment 1.—Two similar capillary pipettes—A and B—are taken. The stems are marked off into divisions of equal length. By the aid of a teat, 6 per cent. salt solution is then aspirated into each—the inflow being arrested when fluid comes level with the fiducial mark in the neck of the stem. The ends of the capillary stems having been sealed, there is now, in pipette A, imposed upon the salt solution a measured volume of a broth culture of staphylococcus mixed with an equal volume of coloured water. In pipette B there is imposed upon the salt solution the same quantity of staphylococcus mixed with an equal volume of coloured serum.

The tubes are now set aside for ten minutes. We then take them in hand,

and in each case cut across the stem at the upper fiducial mark, and then, using for the purpose pipettes drawn out into hair-fine stems, empty out from pipette A and pipette B the contents of the capillary stem—compartment by compartment, planting out as we go upon nutrient agar.

It will be found that in pipette B—that in which infected serum was superimposed upon the salt—the microbes have been carried down to the very bottom of the capillary stem. In pipette A—that in which an infected watery fluid was superimposed—the microbes will have gravitated down only a very short way.

Experiment 2.—Two similar capillary pipettes, with stems divided off into segments of equal length, are taken. A coloured mixture, consisting of nine volumes of water mixed with one of staphylococcus culture, is introduced by capillarity into the distal extremity of the one; and a similar quantity of a coloured mixture of nine volumes of serum and one of staphylococcus culture into the other, the inflow being arrested when the fluid reaches the first division mark. Then, in each case, the distal end of the pipette is turned up sufficiently to displace the column of fluid, and to bring it into position a little distally to the antepenultimate fiducial mark. The tips of the tubes are now sealed up in a by-pass flame. Then, by means of a capillary pipette drawn out into a hair-fine extremity, and carried down into the stems of the pipettes A and B to a little short of the point where the bacterial fluid is lodged, we—leaving here a bubble of air—fill in, in the case of the pipette B, a 3 per cent. solution of salt; and, in the case of the pipette A, water.

The stems having been thus filled in, we take in each case a solid-ended hair-fine glass thread (obtained by drawing out in the flame a piece of capillary tube and fusing its end), and thrust it into the stem of the pipette, carrying it down until it enters the bacterial fluid, and pushes this up level with the penultimate fiducial mark. By the aid of the glass thread, the fluid in the upper part of the stem is let down quite gently upon the bacterial fluid—the intervening bubble of air mounting up the while to find escape in the neck of the pipette. We now set aside the pipettes for twenty-fours hours, and then emptying the compartments one by one from above downwards, plant out the contents upon nutrient agar.

The cultures so obtained show that, where salt solution is superposed upon infected serum, the microbes are carried some distance up the stem; while in the case where we have water superposed upon microbes suspended in water, they are confined to the distal end of the stem.

It thus is manifest that we have in an hypertonic solution an agent which is capable of drawing out from the cavities and *cul-dè-sacs* of a wound and porous tissues, along with the serum lodged there, the microbes which may be suspended in it. And reflection will show that, inasmuch as we have intertraction between serum and salt solutions, our saline solution will also inevitably be carried into cavities and *cul-de-sacs* and porous tissues.

This being so, it would seem possible, by mixing an antiseptic with salt solution, to convey this also into corners and recesses. But clearly, when thus carried in, the bactericidal efficacy of the antiseptic would depend upon whether it was or was not quenched by the albuminous substances with which it there comes in contact.

Comment.—The body of observations set out above would appear to invite to a re-examination of the doctrine that in *diffusion*—or as it would seem more proper to call it *interfusion*—we are dealing in every case with a perfectly passive recipient fluid and with a disbursing fluid which has a monopoly of activity. In other words, the data here obtained would seem to invite some review of the doctrine that when a solute passes out from its menstruum into an adjoining fluid territory, or a diluting fluid is carried into a concentrated solution, dispersive forces resident in the solute are the only forces which come into operation.

While that doctrine ostensibly holds the field there is to be noted that in German text-books the term *Adhäsion,* and in French text-books the term *appel* are still employed in explanation of the passage of water through a dialysing membrane into salt solution. In view of the observations here set out it may perhaps be legitimate to put forward for consideration whether the term *Adhäsion* (which would have as its English equivalent "binding or conjoining force") and the term *appel* (which might perhaps be translated into the invocation "come hither") are simply a figure of speech, a figure behind which there lurks nothing substantial and objective.

And if it be permissible to generalise from the case of what happens when albuminous substances and saline solutions are brought into conjunction, it may be suggested that it would be appropriate explicitly to recognise the existence of *tractor* or *drawing forces,* and more generally of *intertraction* as an agency which may co-operate with diffusion and assist in bringing about interfusion.

On the Local and Generalised Action of Radium and X-Rays upon Tumour Growth.*

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(Communicated by Sir Walter Fletcher, F.R.S. Received December 31, 1920.)

There are many considerations in the successful treatment of a tumour in the animal body by radiation. Broadly speaking, the subject may be divided into two main divisions: (1) the action of X-rays and the beta- and gamma-rays from radium on the tumour cells, and (2) the effect of these rays upon the animal itself.

In both cases a gradual change of effects is observed according to the dose of radiation. Actively growing malignant cells, given a large dose of radiation, degenerate and die when re-inoculated into a living animal; a dose short of this quantity causes the tumour cells to grow at a slower rate than they would do normally, but if given a very small dose, the cells appear to be stimulated rather than hindered in their subsequent growth.

Prolonged exposure of the animal to the rays results in severe wasting and death; with a reduction in the exposure a growing animal will retain its health, but with a diminished rate of increase of body weight. As the dose of radiation becomes less, a stage is reached when the rate of increase of body weight exceeds that of the normal animal; and, when the animal is given these very small doses of X-rays, it is found to develop a state of increased resistance to an implanted tumour.

Such briefly are the chief effects to be considered when devising methods by which a lethal dose of radiation may be given to a tumour without reducing the resistance of the animal.

The experimental data in this paper will be dealt with as follows:—

(A) The effect of the rays in various doses upon malignant cells, before inoculation.

(B) The effect of the rays in various doses upon normal animals—(1) body growth, and (2) subsequent inoculations of malignant cells.

(C) The effects of the rays in various doses upon animals which are bearing tumours.

This investigation has been carried out upon rats, and the tumours have been of three distinct types: Jensen's rat sarcoma; a very slowly growing rat sarcoma (F. 16), for which we are indebted to Dr. J. A. Murray, the Director of the Imperial Cancer Research Fund; and a rat carcinoma, which occurred as a spontaneous tumour in these laboratories in January, 1919, and which has been described (1) to the Pathological Society of Great Britain and Ireland, March, 1920. Most of the work has been done with Jensen's rat sarcoma, and, except where otherwise stated, the observations refer to this tumour.

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* This investigation includes, though is not restricted to, experimental work undertaken at the request of the Medical Research Council upon the general biological effects of small doses of X-rays.

(A) *The Effect of Rays in Various Doses upon Malignant Cells.*

A dose of X-rays or radium rays can be given most accurately to malignant cells when the tumour is not in the animal body. The method we have adopted since 1912 (Wedd and Russ, 2) has been to excise a tumour, cut a slice of it, and expose this aseptically to radiation for any length of time desired; the tumour cells are then implanted into normal rats, and the size of the subsequent growth is accurately recorded. It is more convenient in this type of experiment to use the beta-rays from radium than X-rays, for, however carefully the X-rays may be controlled, there is much more likely to be a change in the radiating power of the source than if radium is used.

Table I shows the results which are obtained when the cells of Jensen's rat sarcoma, before being inoculated into normal animals, receives exposures ranging from forty minutes down to twelve seconds. The last column compares the size of the irradiated with the non-irradiated tumour in the same animal; the inoculations were made in the right and left axillæ. The source of the rays used was a capsule containing 20 mgrm. of radium bromide ($Ra.Br_2.2H_2O$), spread over an area of 4 sq. cm., and covered with a thin layer of varnish; the varnish absorbs alpha- and soft beta-rays.

Table I.

Time of exposure to radium.	Number of rats in which the irradiated tumours did *not* grow.	Number of rats in which the irradiated tumours *did* grow.	Volume of growth compared with control 3–4 weeks after inoculation.
40 minutes.........	12	—	—
35 ,, ...	11	1	0·07
30 ,, ...	13	5	0·29
10 ,, ...	—	6	0·44
5 ,, ...	—	6	0·85
1 minute	—	8	0·82
12 seconds	—	8	1·35

The conclusion drawn from this series is that, as the dose of rays given to the sarcoma cells diminishes from a lethal dose, a gradual change is observed.

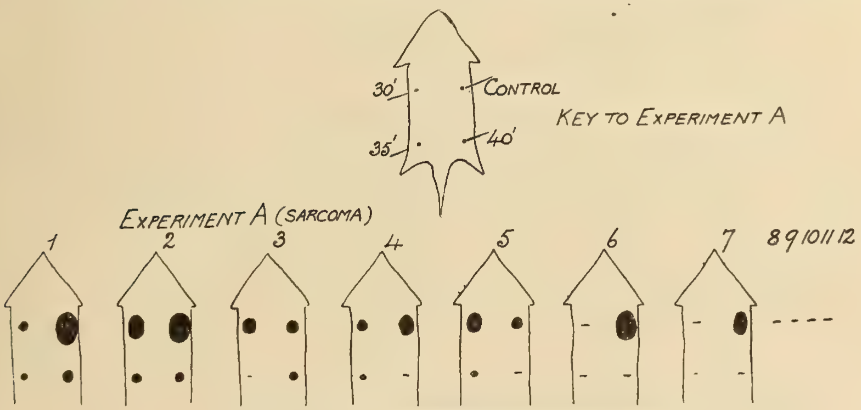
A reduced rate of growth changes into a rate which appears slightly quicker than that of the untreated tumour. This latter phenomenon will be referred to again in Section C.

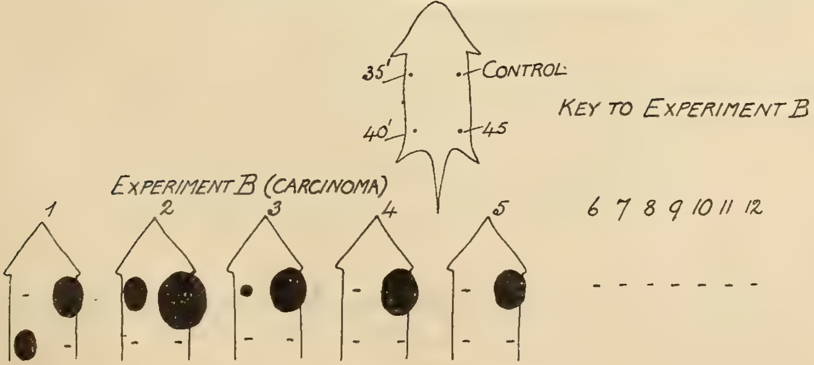
It will be seen from the data in Table I that the lethal dose is about thirty-five minutes, only one animal out of twelve bearing a tumour, when the cells had previously been irradiated; normal tumour inoculated into the left axillæ of the same animals grew in every case.

The lethal dose for the two other varieties of tumour already referred to has been found by following a similar method to that just outlined.

It is not possible to express the results of these experiments in quite the same way as for the Jensen's tumour, because they do not give 100 per cent. of growing tumours when inoculated. The rat carcinoma is very difficult to propagate, rarely as many as 50 per cent. of the inoculations into normal animals resulting in tumours.

The subjoined two charts illustrate the results obtained upon two series of animals. The tumours depicted in the charts are drawn to scale, the time being fifty-six days and twenty-seven days respectively after inoculation.


30'
Control
35'
40'
Key to Experiment A
Experiment A (sarcoma)
1
2
3
4
5
6
7
8 9 10 11 12


35'
Control
40'
45
Key to Experiment B
Experiment B (carcinoma)
1
2
3
4
5
6 7 8 9 10 11 12

In the case of the slowly growing sarcoma, Experiment A, it will be seen that, in three animals out of seven, grafts of the tumour, irradiated for forty minutes, formed very slowly growing tumours; the lethal dose thus appears to be rather larger than for the Jensen tumour or for the carcinoma, Experiment B.

In this connection we may recall the results of Wood and Prime (3); these authors observed only small differences in the dose of beta- and gamma-rays required to prevent growth of four different types of tumours in the mouse and rat.

The dose of radiation needed to prevent any particular tumour growing depends, therefore, not only upon the type of tumour, but also upon the susceptibility of the animal into which the irradiated cells are implanted. The figure made use of, in referring to the lethal dose, is the upper limit given by the highly susceptible animal.

(B) *The Effect of the Rays in Various Doses upon Normal Animals.*

(1) Body weight.
(2) Resistance to subsequent inoculations of malignant cells.

(1) *Body Weight.*—The rays used were X-rays from a Coolidge tube run at an alternative spark gap of 4·5 cm. between spheres 5 cm. diameter. The animals were placed 20 cm. below the anti-cathode, the rays being unscreened except for a thin mica sheet below the tube and a perforated cardboard cover of the animal box. By slightly tilting the tube, a lateral beam of the X-rays used passed from the exposure room through a hole in the wall across a passage into an adjoining room, where its intensity was measured by a gold-leaf electroscope 6 metres distant. By adjusting the radiation to give a standard rate of movement of the gold-leaf, before, and where possible during the exposures, a useful check was kept on the constancy of the radiation used. Under these experimental conditions, daily doses of X-rays were given to batches of animals for a number of weeks, and their body weights compared with those of an equal number of normal animals of the same initial weight. Exposures of five minutes per day were found to be injurious to the animals, so the daily doses were reduced to one minute, in the next batch to twelve seconds, then to two seconds; the effects of this repeated radiation are summarised in Table II.

It will be seen that a daily exposure of one minute is deterrent to increase of body weight; with a diminished dose, there is a more rapid increase than in normal animals.

Table II.

Number of X-rayed animals.	Exposure to X-rays.	Effect upon body weight.
16	1 minute daily for 9 weeks	*Result*: 25 per cent. diminution below normal rate of growth in 63 days. (All but 4 died.)
22	12 seconds daily for 8 weeks	*Result*: 15 per cent. increase above normal rate of growth in 62 days.
21	12 seconds daily for 4 weeks	*Result*: 7·5 per cent. increase above normal rate of growth in 50 days.
23	2 seconds daily for 9 weeks	*Result*: 10 per cent. increase above normal rate of growth in 69 days.

(2) *Resistance to Subsequent Inoculations of Malignant Cells.*—It has been recorded (4) that rats exposed to small daily doses of X-rays before inoculation were found to have a greater resistance to the growth of sarcoma than normal rats. This immunity was not absolute, for many of the rats grew tumours, though smaller ones than the controls. Further experiments have been carried out to determine whether complete immunity could be produced by varying the period over which the irradiation was continued, the actual daily dose of 12 seconds being unaltered.

The data in Table III show the rate of growth of Jensen's rat sarcoma in animals treated in this way. In all cases in which rats have been previously submitted to X-rays the tumours grow at a slower average rate than in the normal animal. It has not been found possible to confer an absolute degree of immunity in this way, but it should be mentioned that we are dealing with a very rapidly growing tumour, one that doubles its volume in less than four days.

It appears from these results that the most prolonged time of irradiation does not give the highest degree of immunity.

In contrast with this increase in the resistance of the susceptible animal we have the experiments of Murphy and Morton (5), of Mottram and Russ (6), and of Prime (7), who showed that, after a very large dose of X-rays, an immune animal becomes temporarily susceptible to carcinoma and sarcoma inoculations.

The effect of a single rather large dose of X-rays in increasing the susceptibility of the animal to inoculation has also been shown by the authors (1), in the propagation of a spontaneous rat carcinoma.

Table III.

Period of irradiation.	Interval before inoculation.	Number of animals.	Volume of tumour compared with that of controls.			Remarks on tumour growth.
			Time after inoculation.			
			2 weeks.	3 weeks.	4 weeks.	
4 weeks	28 days	18 X-rayed 26 controls	0·77	0·46	0·40	X-rayed animals grew 4 progressive tumours. 14 disappearing tumours. Control animals grew 12 progressive tumours. 14 disappearing tumours.
6 weeks	13 days	9 X-rayed 10 controls	0·65	0·44	—	X-rayed animals grew 3 progressive tumours. 6 disappearing tumours. Control animals grew 9 progressive tumours. 1 disappearing tumour.
8 weeks	11 days	21 X-rayed 20 controls	0·42	0·40	0·35	X-rayed animals grew 7 progressive tumours. 14 disappearing tumours. Control animals grew 14 progressive tumours. 6 disappearing tumours.
12 weeks	13 days	29 X-rayed 24 controls	0·84	0·76	0·65	X-rayed animals grew 19 progressive tumours. 10 disappearing tumours. Control animals grew 17 progressive tumours. 7 disappearing tumours.

(C) *The Effect of the Rays in Various Doses upon Animals which are Bearing Tumours.*

Here we have to distinguish between three varieties of exposure:—

(1) Exposures in which the animal does not share to an appreciable extent in the radiation that the tumour receives, *i.e.*, a localised exposure to beta-rays.

(2) Exposures in which the tumour does not share in the irradiation of the animal, *i.e.*, tumour screened from the rays.

(3) Exposures in which the animal shares in the radiation that the tumour receives, *i.e.*, a generalised exposure of X-rays to the whole body.

(1) *Localised Exposures.*—If a small superficial tumour exists in the animal body, it is possible, by the use of easily absorbed rays, such as beta-rays, to expose the cells of the tumour without the rest of the animal sharing to an appreciable degree in the radiation.

A series of experiments upon a number of animals was carried out on the following lines. About the fourth day after inoculation of a graft, when the tumour was palpable, the radium capsule was held on it for a certain length of time. The minimum time of exposure necessary to prevent growth of the tumour was found; it was not appreciably different from the exposure required if the tumour material was removed from the body, given its dose of radiation and re-inoculated into susceptible animals in the manner described in Section A. The results were analogous indeed to those given in Table I to the extent of showing that a small dose of radiation given to the tumour, increased rather than hindered its growth.

With the tumour in the animal, these exposures to small quantities of radiation may be repeated in a way not possible when the tumour is irradiated outside the body.

In the next series of experiments, the rats were inoculated on each side, four days later all the tumours on the right sides were exposed to the radium for times varying from ten minutes to twelve seconds; these doses were given once a week, their effect upon the growth of the tumour is seen in Table IV.

Table IV.

Conditions of irradiation of tumour in animal.	Volume of irradiated tumours compared with the volume of controls in the same animal.				Remarks.
	1 week.	2 weeks.	3 weeks.	4 weeks.	
15 rats, 10 minutes' exposure to beta-rays once a week	0·59	0·43	0·42	0·44	In 13 rats the control tumour was the larger.
15 rats, 5 minutes' exposure to beta-rays once a week	0·92	0·76	0·76	0·85	In 13 rats the control tumour was the larger.
19 rats, 1 minute's exposure to beta-rays once a week	0·98	1·07	1·25	1·41	In 14 rats the irradiated tumour was the larger.
17 rats, 12 seconds' exposure to beta-rays once a week	1·14	1·28	1·33	1·46	In 10 rats the irradiated tumour was the larger; in 5 rats the control; and in 2, both tumours were the same size.

The general trend of these observations is that, while a weekly exposure of ten or five minutes checks the tumour growth, a reduction to one minute or less has an opposite tendency. The beta-rays used were those from the capsule employed in the experiment detailed under Section A (Table I). The results show that a dose of radiation of about one-fiftieth of the lethal dose (thirty-five or forty minutes, *vide* Table I), has, if anything, an accelerating effect upon tumour growth.

(2) *Generalised Radiation of the Animal, the Tumour being Screened.*—A number of animals (twenty-four) were inoculated with the sarcoma, in the middle of the back. The next day twelve of the animals were submitted to a daily 12-second dose of X-rays; the graft was covered with a piece of lead 2 mm. thick, to screen it from the X-rays. These exposures were continued daily for a month, measurements of the tumours being made twice a week. A comparison with the tumours in the twelve control animals is made in Table V.

Table V.

Number of animals.	Time after generalised radiation began.	Volume of X-rayed tumours compared with controls.	Remarks.
12 X-rayed	1 week	0·80	X-rayed animals grew 6 progressive tumours. 6 disappearing tumours. Controls grew 10 progressive tumours. 2 disappearing tumours.
	2 weeks	0·74	
12 controls	3 weeks	0·56	
	4 weeks	0·55	

The result is similar to those in Section B (2), Table III, in which a decreased susceptibility to tumour growth follows a generalised irradiation.

(3) *Generalised Exposures.*—From the preceding observations, it will be clear that we have to deal with a positive and a negative factor; for if, on the one hand, we have shown that a repeated exposure of the animal to small doses of X-rays *increases* its resistance to tumour growth, we have to recognise, on the other hand, that the effect of small doses of radiation upon the tumour cells is rather to accelerate their rate of growth. It remained for experiment to decide which process would outweigh the other when a tumour-bearing animal was given a generalised radiation repeated at frequent intervals.

Two batches of animals were inoculated in the right axilla; one batch received a daily dose of X-rays, the other no radiation. The dose of X-rays, in different experiments, was varied from five minutes to two seconds. The growth of the tumour in the two batches was then recorded for a period of about one month; the results obtained will be seen in Table VI.

The effect of five minutes' exposure to X-rays daily for one week is to retard the tumour growth, probably by direct action upon the cells, but it has at the same time a profound effect upon the animal, causing death in nearly every case. A reduction of the exposure to one minute again results in reduced rate of tumour growth, but the body weight suffers in comparison with the non-irradiated animal. Further reduction of the exposure has less effect in slowing up the tumour, though the body weight has

Table VI.

Number of animals.	Time of exposure to X-rays.	Growth of tumour in X-rayed animals compared with controls. Weeks after inoculation. 1	2	3	4	General Remarks. Tumour growth.	General condition.
12 X-rayed 12 controls	5 minutes daily for 1 week	0·13	0·03	0·01	—	No progressive growth of tumour in the X-rayed animals	All X-rayed animals except 4 died within 4 weeks, 1 survived 3 months.
23 X-rayed 19 controls	1 minute daily for 4 weeks	1·0	0·76	0·72	0·36	X-rayed animals grew 13 progressive tumours 10 disappearing tumours Controls grew 15 progressive tumours 4 disappearing tumours	X-rayed animals well, but body weight increased 19 per cent. less than the normals.
14 X-rayed 15 controls	12 seconds daily for 4 weeks	0·81	0·73	0·82	0·73	X-rayed animals grew 9 progressive tumours 5 disappearing tumours Controls grew 11 progressive tumours 4 disappearing tumours	X-rayed animals well, no record of body weight, but *vide* Table II, rows 2 and 3.
36 X-rayed 33 controls	2 seconds daily for 4 weeks	1·25	1·23	1·05	0·79	X-rayed animals grew 17 progressive tumours 19 disappearing tumours Controls grew 20 progressive tumours 13 disappearing tumours	Animals well, body weight increase same as normals.

increased more than normally. When the tumour growth figure 0·73 at the fourth week is compared with the figure 0·55 at the fourth week in Table V, it seems rational to attribute this difference to the slightly stimulating effect which small doses have been shown to have upon the malignant cells (*vide* Tables I and IV).

Discussion of Results.

Since the attempt has been made throughout these investigations to express the results quantitatively, it remains to consider to what extent the observed differences in the rate of growth of the animals and of the tumours are significant. In Section A, the matter which calls for comment is that the rate of growth of tumour was 1·35 times that of the normal when the tumour cells had previously received a small dose of beta-rays. The number of animals used, viz., eight, is not large enough to consider the matter established, but, when the experiment is done under slightly different conditions with the same dose of rays, a similar increase occurs in the rate of growth of tumour, viz., 1·46 times the normal, the number of animals being seventeen (*vide* Table IV).

The data in Section B (Table II) show that the body weight of the rat increases 15 per cent. more rapidly under small dose X-ray treatment than that of untreated animals of the same initial body weight; the number of animals used is enough to preclude the effect being accidental.

The increased resistance to tumour inoculations of an animal which has previously been given small daily doses of X-rays is a fairly constant feature of Table III. Considering the number of animals used (viz., 77, and an equal number of controls), it is very improbable that the reduction in tumour growth, amounting on the average to about 40 per cent., can be attributed to anything but the X-ray treatment.

In Section C, Table V, the quantitative reduction in the rate of tumour growth appears to be sufficiently large to preclude the element of chance. There is less restraint upon the growth of the tumour when the tumour itself is allowed to share in the small-dose radiation treatment (*vide* data in Table VI).

The bearing of these investigations upon the radiological treatment of malignant disease in man appears to be two-fold. In the first instance it must be recognised that the uniform irradiation of a large tumour in the human subject is hardly possible whether X-rays or radium be used. Small variations from the lethal dose would appear unimportant, but should the quantity of radiation reaching outlying portions of the growth be so diminished in intensity as to be a *small dose* (such as a few per cent. of the lethal dose) it might have a stimulating instead of a destructive effect on the malignant cells. Turning now to the body as a whole, there seems ample evidence to show that large generalised doses of radiation lower the normal resistance to tumour growth. This result is completely reversed when the normal animal is given very small generalised doses of X-rays, repeated at frequent intervals, and it would therefore seem a rational measure to supplement the local intensive irradiation of a tumour by a feeble generalised irradiation of the patient, care being taken wherever possible not to expose the tumour cells to this radiation.

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The Enzymes of B. coli communis. Part V.—(a) *Anaerobic Growth followed by Anaerobic and Aerobic Fermentation.* (b) *The Effects of Aeration during the Fermentation.*

By E. C. Grey and E. G. Young.

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In the present communications the effect of oxygen upon the fermentation of glucose and upon the growth of the bacteria, in so far as this affects fermentation, is considered. To this end the organisms have been grown both aerobically and anaerobically, and subsequently made to ferment glucose, both aerobically and anaerobically, with the object of comparing the products of decomposition in the two cases. There are clearly two problems: firstly, the effect of exposure to oxygen during growth upon the subsequent fermentation, whether aerobic or anaerobic, and, secondly, the effect of oxygen admitted during the fermentation. The first question relates to the part played by oxygen in the formation of enzymes, the second to the part played by oxygen in their action on carbohydrates. The first question is considered, though in but a preliminary way, in Section A, the second, more fully, in Section B.

SECTION A.

Object of the Experiments.

Two results were aimed at in these experiments. Firstly, to compare the products of fermentation of glucose anaerobically, after anaerobic growth, with the products of fermentation anaerobically after previous growth aerobically. And, secondly, to obtain information as to the effect of introducing oxygen during the fermentation itself. This latter consideration, however, though brought to notice by these experiments, is considered only incidentally here because it forms the subject of Section B. In the present section we wish to direct attention particularly to those differences which exist between the fermentation after anaerobic and aerobic growth, not upon the effect of aeration during the fermentation. To point out the difference which previous growth aerobically or anaerobically has made, several analyses from previous experiments are included in Table IV side by side with the completely anaerobic experiments of Tables I, II, and III.

TECHNIQUE.

Growth of the Organisms anaerobically prior to Fermentation.

Winchester quarts containing agar were inoculated with *B. coli communis* which had been grown anaerobically on broth in an atmosphere of hydrogen. The procedure was to sterilise the whole apparatus, consisting of Winchester quart containing agar and side tube containing broth. The apparatus was cooled in nitrogen, and when cold the side tubes containing broth were inoculated. The bottles were placed side by side in a large incubator and connected in series; hydrogen was then passed through the whole series, and in this way the broth in the side tube of each bottle was anaerobic during the growth of the bacteria. Strict anaerobiosis was maintained by exhausting at the pump from time to time and allowing fresh hydrogen to enter. After seventy-eight hours the broth of the side tubes was allowed to run into the bottles and the growth on agar continued for forty-eight hours. To remove the bacterial growth, a vacuum was created in the bottles and saline solution, such as has been used in all previous experiments of this series, was allowed to enter. The saline emulsion of bacteria was removed to the fermentation flask by previously exhausting the latter of air and allowing the hydrogen to enter the Winchester quarts. The hydrogen was subsequently pumped out of the fermentation flasks by boiling the contained fluids *in vacuo*, and nitrogen was admitted. The fermentation was allowed to proceed anaerobically, so that the whole of the operation of growth of the bacteria and of fermentation by them of the sugar was carried out with rigorous exclusion of air. The operations were found exceedingly difficult.

As regards the fermentations themselves, the technique was that described in Part III of this series, with the addition of an arrangement for regulating the admission of oxygen or air at a measured rate where aerobic fermentations were desired.

Experiment I.—The bacteria were grown anaerobically on agar and approximately equal portions of bacterial emulsion were added to each of three flasks containing sugar, water, and some chalk. The fermentation was allowed to continue forty-eight hours at 37° C. Experiment N was completely anaerobic, A received two litres of air, O two litres of oxygen during the fermentation.

The analysis of the fermentation products gave the following results, expressed as percentages upon the sugar employed:—

Table I.—Fermentation of Glucose by *B. coli communis* grown anaerobically. The fermentations carried out in Nitrogen (N), Air (A), and Oxygen (O), respectively.

Products.	Products as percentages of glucose employed.		
	N.	A.	O.
Hydrogen	0·61	0·50	0·40
Carbon dioxide	27·33	24·17	20·00
Formic acid	16·68	12·33	0·08
Acetic acid	8·67	9·50	18·15
Succinic acid	9·83	10·33	12·33
Lactic acid	Nil	12·00	8·83
Alcohol	7·33	8·66	9·00
Total	70·45	77·49	68·19

It will be seen that there is a gradual diminution of CO_2 production with increasing oxygenation, and a still greater diminution of formic acid. The effect of admitting oxygen during the fermentation, however, will be discussed more fully in Section B of this communication.

Most striking of all is the absence of lactic acid from the completely anaerobic fermentation, and the reappearance of this acid with aeration. It is possible that the missing 30 per cent. of glucose has in part been converted into some mother substance of lactic acid. In Part III of this series an experiment is described in which samples of the fermentation fluid were removed for analysis every twelve hours. At the end of the first period of twelve hours which had been one of rapid death of the bacteria introduced, no lactic acid was found, in the second twelve hours although the glucose disappeared as evidenced by Fehling's Solution, the products recovered only represented a small fraction of the glucose consumed, but in the third period, from twenty-four to forty-eight hours, all the missing glucose reappeared as lactic acid. It would thus seem that the transformation of glucose into some non-reducing carbohydrate is a preliminary reaction to lactic acid formation, and this probably accounts for what has happened in Experiment I.

The results of Table I are represented pictorially in fig. 1A.

Experiment II.—The bacteria were grown anaerobically as before. In B the fermentation was completely anaerobic while in C air was admitted.

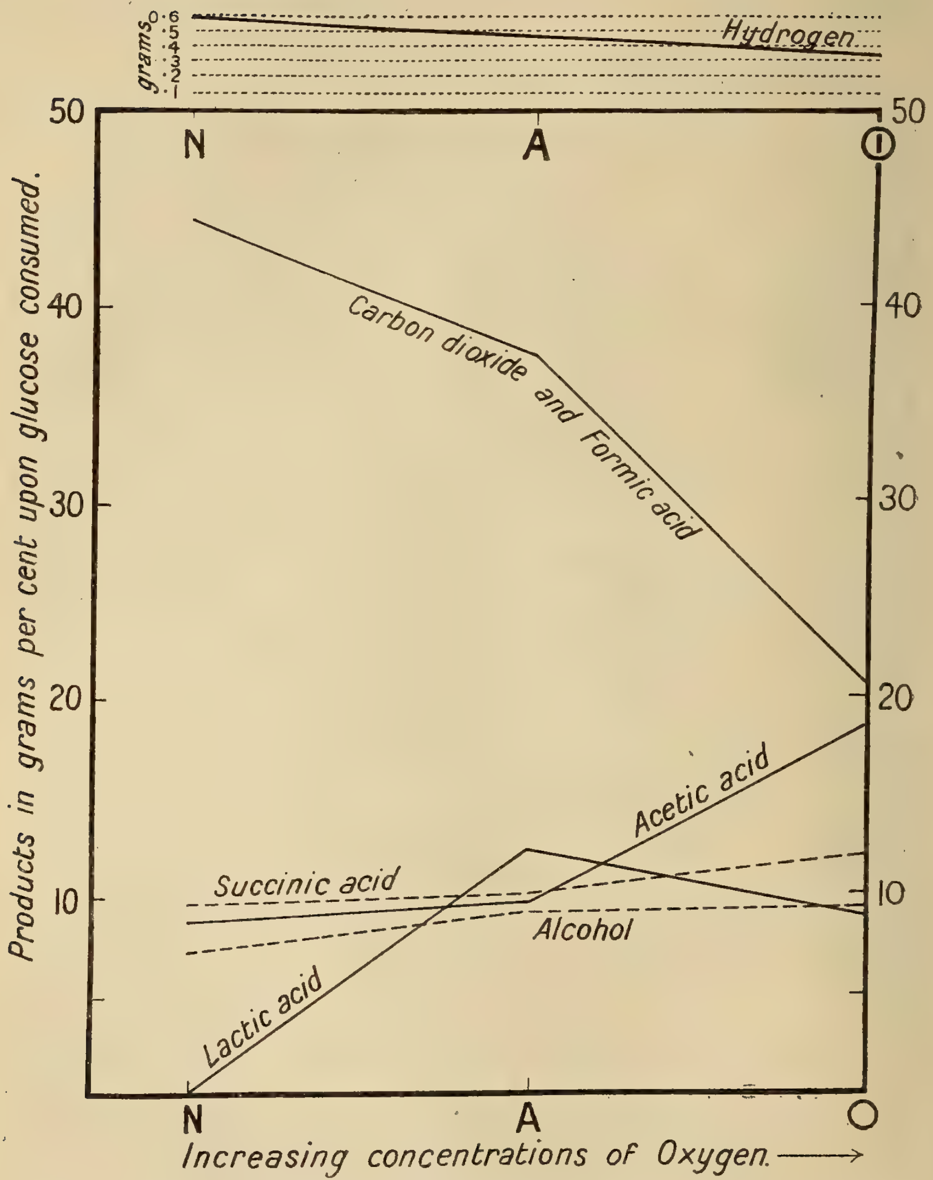

FIG. 1A.—Effect of oxygen upon fermentation of glucose by *B. coli communis*, grown anaerobically; fermentation carried out in nitrogen (N), air (A), and oxygen (O).

In this case some lactic acid has been formed, but when it is recollected that normally this bacterium produces from 20 to 30 per cent. or more of lactic acid it will be seen that the previous anaerobic growth has led to a very marked diminution in lactic acid production. It is to be noted also that succinic acid has been markedly diminished, even more so than lactic acid and in the place of both acetic acid appears.

Table II.—Fermentation Anaerobic (B) and Aerobic (C) after Anaerobic Growth.

Products.	Products expressed as percentages of the glucose consumed.	
	B.	C.
Hydrogen	Not estimated.	Not estimated.
Carbon dioxide	27·30	26·42
Formic acid	12·64	4·96
Acetic acid	34·38	44·76
Lactic acid	8·44	8·20
Succinic acid	0·58	2·46
Alcohol	15·66	12·36
Total	99·00	97·16

In Experiment II a very large amount of bacteria was introduced and the aeration during the fermentation seems to have made but little difference. A third experiment was made similar to the previous one but with less emulsion of bacteria. D was fermented anaerobically; E received a little air at the beginning of the fermentation.

Table III.—Fermentation Anaerobic (D) and Aerobic (E) after Anaerobic Growth.

Products.	Percentages of products upon the glucose consumed.	
	D.	E.
Hydrogen	0·36	0·18
Carbon dioxide	37·32	23·03
Formic acid	8·75	5·18
Acetic acid	41·07	57·68
Lactic acid	1·25	2·68
Succinic acid	Nil	Nil
Alcohol	10·00	5·00
Total	98·75	93·75

Here again lactic acid is practically absent as in Experiment I, and succinic acid is completely absent, confirming Experiment II. Thus two striking phenomena are to be observed when the fermentation of glucose is carried out by *B. coli* previously grown anaerobically; the one is the absence of lactic acid and the other the absence of succinic acid from the products of decomposition. In place of them acetic acid appears.

In order to make clearer the effect which aerobic and anaerobic growth has upon the subsequent fermenting power of the bacteria, Table IV is included wherein the anaerobic fermentations described in this communication are compared with typical anaerobic fermentations which have been carried out with bacteria grown aerobically as described in Parts II, III and IV of this series.

Table IV.—A Comparison of the Products of the Anaerobic Fermentation of Glucose by *B. coli*, grown previously Anaerobically and Aerobically.

Products.	Products as percentages of the glucose consumed.*						
	Bacteria grown aerobically.			Bacteria grown anaerobically.			
Hydrogen	0·33	0·21	0·35	0·61	—	0·36	0·18
Carbon dioxide	16·37	15·24	19·38	27·33	27·30	37·32	23·03
Formic acid	2·76	12·29	5·22	16·68	12·64	8·75	5·18
Acetic acid	19·34	22·02	18·56	8·67	34·38	41·07	57·68
Lactic acid	28·47	25·46	16·93	Nil	8·44	1·25	2·68
Succinic acid	20·32	11·23	17·36	9·83	0·58	Nil	Nil
Alcohol	11·04	13·36	20·06	7·33	15·66	10·00	5·00
Total	98·63	99·81	97·86	70·45	99·00	98·75	93·75

* Fermentations all anaerobic.

Note on the Relation of Oxygen to the Growth of B. coli communis.

The organism we have been using has frequently been described as a facultative anaerobe. It is easy to see that the growth is much more extensive on agar in air than in hydrogen. Under comparable conditions we obtained 70 mgrm. of *B. coli* (dry weight) after anaerobic growth on agar, and 280 mgrm. after aerobic growth. In the absence of oxygen, *B. coli* grows better in a fluid nitrogenous medium than on an agar surface. It has already been shown (Part III) that when an emulsion of bacteria is mixed with glucose solution the bacteria while carrying out a rapid fermentation of the glucose (1 grm. per hour per gramme of bacillus) are rapidly dying, and in this death process no lactic acid is formed. Subsequently a new generation of bacteria replaces the old and these give rise to lactic acid. *B. coli* would thus appear to have two distinct forms of metabolism. When the organisms have had an immediate past history of life in air they require oxygen for their continued existence in this form; if they must ferment anaerobically they die rapidly in doing so, but some of the individuals adapt themselves to the anaerobic conditions and probably this means that they possess a mechanism for using combined oxygen. This is discussed later. The results described in this communication must be considered in the light of these remarks for doubtless the increase of lactic acid following admission of oxygen in Experiment I is to be referred to an increased vitality of the bacterial culture, *i.e.*, either the bacteria increased in numbers, or they took longer to die. On the other hand it may be that oxygen is necessary under certain circumstances for the action of the enzyme mechanism which produces lactic acid, but this seems unlikely for reasons stated in the discussion later.

Discussion of Results.

B. coli communis grows better in air than in an atmosphere of hydrogen and ferments glucose more vigorously anaerobically after growth in air than after anaerobic growth. The type of fermentation differs according as the immediate past history of the organism has been aerobic or anaerobic. In the latter case, no lactic acid or only a very slight yield is obtained, and also succinic acid tends to be absent.

The manner in which oxygen affects the fermenting powers of the bacteria subsequently is not obvious, but it seems likely that the oxygen has acted as a stimulus to reproduction, so that when subsequently fermentation takes place multiplication of cells occurs, whereas when this stimulus is absent, as in the anaerobic past history, the fermentation occurs without such reproduction. This view accords well with the observations recorded in Part III of this series where only during a period of cell multiplication was lactic acid produced.

Oxygen in the free state is not necessary for lactic acid production if the bacteria are allowed to multiply in a nitrogenous medium such as peptone, but in the case of bacteria grown previously anaerobically on agar the stimulus of oxygen seems necessary for the production of lactic acid. It is probable that the oxygen acts by increasing cell multiplication, but this has not been proved.

Summing up then, lactic acid production is associated with rapid multiplication of cells, and the cause of lactic acid increase when oxygen is admitted is probably due to stimulation of growth.

Death of the cells and conditions of depressed vitality are associated with the production of carbon dioxide and acetic acid or alcohol.

These results may possibly explain the phenomenon of increased growth of yeast due to preliminary aeration. Horace Brown put forward as an explanation the idea that oxygen was stored up, and subsequent reproduction was proportional to this oxygen charge. But all evidence seems against the idea that any appreciable amount of oxygen is stored up. It is therefore suggested that the effect of oxygen is that of a stimulus to growth and ferment production, and the effect of such stimulus in modifying the subsequent fermentation has been seen in the experiments described in this communication. A little air introduced at the beginning can give rise to a very marked effect upon the whole subsequent course of the fermentation. In the last two experiments described in this communication, namely D and E, the conditions were practically the same, except that by accident air was momentarily admitted to E, there has followed a large increase in acetic acid and diminution of carbon dioxide.

Again it is frequently observed that fermentations which refuse to begin can be made to do so by the introduction of a little air. Pakes has also observed this with formic acid fermentations by various bacteria. Lately we have observed the same, both with formic acid fermentations and with glycerol fermentations.

Oxygen thus appears to be a stimulus to fermentation production by certain bacteria as by yeast. The effect long outlasts the stimulus and is not proportional in any chemical sense to it. This is probably the explanation of the oxygen charge of Horace Brown.

Conclusions of Section A.

Anaerobic fermentation of glucose by an emulsion of *B. coli communis* proceeds differently according as the organisms have been grown previously with or without oxygen.

When the immediate past history has been anaerobic, the fermentation under anaerobic conditions yields very little or no lactic acid and greatly diminishes succinic acid. In place of these, acetic acid appears in large proportion. Admission of oxygen during the fermentation leads to lactic acid production. The fact that acetic acid replaces succinic acid entirely gives additional proof of the close relationship of these two substances.

The results also confirm the conclusions of the earlier sections as to the independence of the lactic acid, acetic acid, and probably of the carbon dioxide fermentations.

SECTION B.

In this Section the effect of admitting oxygen during the fermentation is considered in more detail. Eight experiments are described. In the first four, a solution of peptone and glucose was fermented by the introduction of a small seeding of bacteria. In the second four, an emulsion of bacteria, previously grown on agar, was employed. The eight experiments constitute four pairs, in each of which there is an aerobic and an anaerobic fermentation. In the former case, about 2 litres of air were admitted in forty-eight hours. About 5 grm. of glucose was fermented in each experiment. Further experiments, carried out with more efficient aeration, would be of interest.

Experimental.

In the first four analyses a solution of glucose and peptone was fermented. In A and B the glucose was present when the flasks were inoculated. In C and D the glucose was not added until twenty-four hours after the inoculation of the peptone.

Experiment I.—The solution contained:—peptone, 5 grm.; glucose, 5 grm.; saline,* 500 c.c.; chalk, 5 grm.

After seventy-two hours the glucose was found completely fermented. The results of the analyses are recorded in Table I.

Table I.—Products Expressed as Percentages of Glucose Consumed.

Products.	A (anaerobic).		B (aerobic).	
Hydrogen	0·23	35·70	0·13	24·51
Carbon dioxide	34·22		15·90	
Formic acid	1·25		8·48	
Acetic acid	8·91		17·57	
Lactic acid	10·68		37·57	
Succinic acid	3·94		4·73	
Alcohol	22·45		10·17	
Total	81·68		94·45	

These analyses show that the introduction of oxygen during the fermentation leads to:—diminution of hydrogen, diminution of carbon dioxide, diminution of formic acid, diminution of alcohol;† and to increase of lactic acid and increase of acetic acid.

Experiment II.—A second pair of fermentations was carried out at the same time, and with the same sowing of bacteria as in Experiment I; but the glucose was not added until twenty-four hours from the time of inoculation. Other conditions were precisely the same as in the previous experiment.

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* Saline solution contained 0·6 per cent. K_2SO_4 and 0·1 per cent. $MgSO_4$.

† It is probable that in this analysis it is not that the oxygen has diminished the alcohol, for 10 per cent. is quite an average yield under normal circumstances. Some other factor has led to an increase of alcohol in A above the normal. This large yield of alcohol is evidently to be correlated with the great diminution of formic acid, and it shows that alcohol is formed in relation to the utilisation of hydrogen.

Table II.—Products Expressed as Percentages of Glucose Consumed.

Products.	C (anaerobic).		D (aerobic).	
Hydrogen	0·69	39·74	0·23	18·98
Carbon dioxide	25·83		14·74	
Formic acid	13·22		4·01	
Acetic acid	11·95		43·39	
Lactic acid	19·52		11·69	
Succinic acid	5·61		5·64	
Alcohol	15·34		14·22	
Total	92·18		93·92	

These analyses show that the introduction of oxygen during the fermentation leads to:—diminution of hydrogen, diminution of carbon dioxide, diminution of formic acid; and to increase of acetic acid.

Experiment III.—The next four analyses represent fermentations of glucose by any emulsion of bacteria, previously grown aerobically on agar. E and F were fermented anaerobically, G and H aerobically.

The solutions contained:—glucose, 6 grm.; chalk, 5 grm.; saline, 400 c.c.; bacterial emulsion, 100 c.c.*

Table III.—Products Expressed as Percentages of Glucose Consumed.

Products.	E (anaerobic).	F (anaerobic).	G (aerobic).	H (aerobic).
Hydrogen	0·49	0·41	0·31	0·18
Carbon dioxide	16·19	17·28	6·55	13·12
Formic acid	10·74	11·01	7·02	5·57
Acetic acid	21·11	9·50	16·74	11·49
Lactic acid	23·16	20·33	25·24	30·23
Succinic acid	11·87	11·49	18·23	16·30
Alcohol	9·06	10·30	10·41	10·17
Total	92·62	90·32	84·50	87·06

These analyses show that the introduction of oxygen during the fermentation leads to:—diminution of hydrogen, diminution of carbon dioxide, diminution of formic acid; and to increase of lactic acid and increase of succinic acid.

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* Emulsion contained 0·1 grm. bacteria (dry weight) and 0·3 grm. soluble organic matter.

In Table IV, these results are put together in order from left to right of decreasing values for CO_2 + formic acid. For the moment, we wish to direct attention merely to those changes which have resulted from the introduction of air during the fermentation; changes due to other factors we will discuss later.

Table IV.—Products of the Decomposition of Glucose by *B. coli communis* Expressed as Percentages of Glucose Consumed. Experiments arranged from left to right in order of decreasing values of (CO_2 + Formic Acid).

Products.	C.	A.	F.	E.	B.	D.	H.	G.
Hydrogen	0·69	0·23	0·41	0·49	0·13	0·23	0·18	0·31
Carbon dioxide	25·83	34·22	17·28	16·19	15·90	14·74	13·12	6·55
Formic acid	13·22	1·25	11·01	10·74	8·48	4·01	5·57	7·02
Acetic acid	11·95	8·91	19·50	21·11	17·57	43·39	11·49	16·74
Lactic acid	19·52	10·68	20·33	23·16	37·57	11·69	30·23	25·24
Succinic acid	5·61	3·94	11·49	11·87	4·73	5·64	16·30	18·23
Alcohol	15·36	22·45	10·30	9·06	10·17	14·22	10·17	10·41
Total	92·18	81·68	90·32	92·62	94·45	93·92	87·06	84·50

By arranging the products in the order as seen in Table IV, the first thing that is apparent is that all the anaerobic experiments fall on the left, and all the aerobic experiments on the right of the middle line, so that we may say that the most characteristic effect of aeration during fermentation is a diminution in formic acid and the gaseous products, CO_2 and H_2, which for many reasons we have been led to consider are derived from formic acid. Corresponding with this diminution in the products containing one carbon atom there is an increase either of a product with three C atoms (lactic acid) or of two C atoms or a multiple of two (acetic acid, succinic acid). The variations in the products are made clearer by representing the results pictorially as in fig. 1B.

The following points may be noted with regard to fig. 1B:—

(1) The experiments have been arranged in order of decreasing values of CO_2 + formic acid, and no consideration has been taken as to the conditions under which the various experiments were carried out. Nevertheless, it will be seen that all the anaerobic fermentations become grouped on the left and all the aerobic experiments on the right of the figure.

(2) The curve representing alcohol shows very little fluctuation in spite of the great changes in acetic acid, and clearly aeration has but little effect on the alcohol production, though it markedly increases the production of acetic acid.

(3) The two most variable products are lactic acid and acetic acid. And it is remarkable that whereas on the left-hand side of the figure these curves do not cross one another, on the right they do so very frequently. From this it is clear that there is much more constancy between the products derived from glucose by anaerobic than by aerobic fermentation. With aerobic fermentation it would appear that slight variation in the conditions may very greatly affect the resulting proportions between lactic and acetic acid. Bearing in mind what has already been stated in §3 of this series, that variation in the proportions of the products is often to be correlated with variation in the proportion between living and dead or dying cells, it would seem that the most probable explanation of this intercrossing of the curves on the right of the figure is that oxygen has altered this proportion between living and dying cells. And that either the oxygen has caused a multiplication of the cells added in the emulsion, or else at least that it has prevented them dying as rapidly as in the anaerobic fermentations. This is all equivalent to saying that the aerobic fermentations are more complicated by the factor of bacterial life than are the anaerobic fermentation.

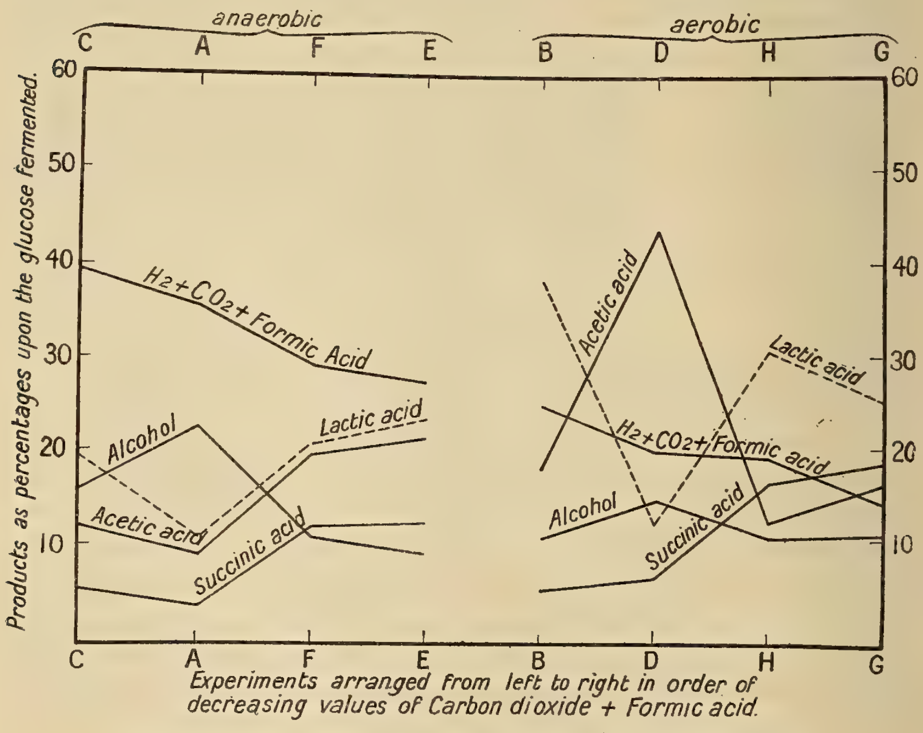

FIG. 1B.—Products of decomposition of glucose by *B. coli communis*.

(4) The figure shows fairly clearly that there are three main lines of

decomposition of the glucose, viz., into products of one, two and three C atoms respectively.

These three groups are primarily represented by formic acid, alcohol + acetic acid, and lactic acid. Secondary reactions may occur, giving rise to succinic acid, or, by an interaction of nascent H from Group 1 with Group 2, the proportion of alcohol to acetic acid may be raised.

In fig. 2 the products of the decomposition of glucose have been plotted as percentages upon the portion of the glucose which has not been turned into lactic acid. That is to say, the lactic acid in each case has been deducted from the weight of glucose consumed, and the products have been recalculated as percentages upon the remainder. The object of this treatment is to do away with variations in the products which result merely secondarily from variations in the lactic acid. One of the immediate results of replotting the products in this manner is to draw attention to the constancy of alcohol under aerobic conditions. It will be seen that in the figures on the left representing anaerobic conditions alcohol shows considerable fluctuations and always in an opposite direction to acetic acid. This relationship has been observed frequently before and can only indicate that the two are derived from the same precursor. The mechanism by which the variations

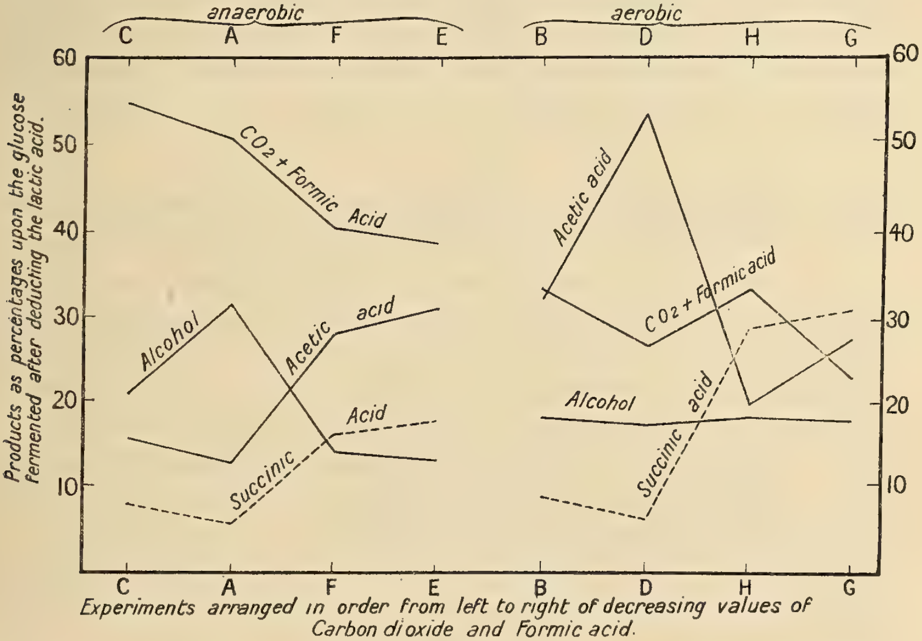

FIG. 2.—Products of decomposition of glucose by *B. coli communis*, after deducting portion changed to lactic acid.

occur depends upon the production of nascent hydrogen, either from formic acid or its precursor. It would appear, therefore, that the effect of oxygen is to inhibit the mechanism for the utilisation of nascent hydrogen. Clearly also, since the total formic acid CO_2 and H_2 is diminished, it is likely that there is less hydrogen available. These experiments would seem to prove conclusively, therefore, that a constant amount of alcohol is produced by the decomposition of *B. coli* on glucose by a fermentation in which nascent hydrogen plays no part, but that an increase in this alcohol production frequently occurs under circumstances where nascent hydrogen is operative. Likewise for acetic acid there is a tendency for a certain constant production in equimolecular proportion to the alcohol, but nascent hydrogen may diminish and oxygen increase its yield. If we try to explain the mechanism by which alcohol and acetic acid arise, we seem to be led again to the Cannizaro reaction.

Calculation of the Oxygen in the Products of Anaerobic and Aerobic Fermentation as compared to the Oxygen in the Glucose fermented.

It is surprising to find that if the oxygen is calculated for each of the products of the fermentation, more is found than corresponds to the glucose fermented, and corresponding with this gain in oxygen, there is a loss of carbon and slight loss of hydrogen. The gain of oxygen and loss of carbon is much more marked in the anaerobic than it is in the aerobic fermentation.

We give the results of two anaerobic and two aerobic experiments calculated out to show these facts in Table V. No account is taken of lactic acid and acetic acid, since these have the same empirical formula as glucose.

As explanation, we can only suggest that water has been added on in the reaction. Further work must be carried out to explain the facts with certainty.

It may be noted that this gain of oxygen and loss of carbon in the products analysed is not observed in the present analyses only, but in all the previous ones. The results given by Harden earlier show the same relationships, also in the cases we have calculated, and, as a matter of fact, in his first paper on this subject, Harden postulated water as taking part in the reactions and entering into the final products.

Conclusions to Section B.

The effect of introducing oxygen in the fermentation of glucose by *B. coli communis* is to increase the lactic, acetic, and succinic acids, and to diminish the hydrogen, carbon dioxide, and formic acid, but to leave the alcohol unchanged.

Table V.—Products of the Aerobic and Anaerobic Fermentation of Glucose by *B. coli communis.* Calculation of the elementary composition of the products to illustrate the gain of oxygen, especially marked in anaerobic fermentations.

Products.	Anaerobic.					
	C.	H.	O.	C.	H.	O.
Hydrogen	—	0·021	—	—	0·018	—
Carbon dioxide	0·189	—	0·503	0·208	—	0·553
Formic acid	0·120	0·020	0·319	0·127	0·021	0·337
Succinic acid	0·207	0·026	0·275	0·206	0·026	0·274
Alcohol	0·202	0·050	0·237	0·237	0·059	0·277
Total	0·718	0·117	1·222	0·778	0·124	1·441
Calculated for glucose	0·822	0·137	1·095	0·890	0·148	1·185
Calculated on basis of the carbon	0·718	0·120	0·056	0·778	0·130	1·037

Products.	Aerobic.					
	C.	H.	O.	C.	H.	O.
Hydrogen	—	0·014	—	—	0·008	—
Carbon dioxide	0·080	—	0·215	0·161	—	0·426
Formic acid	0·082	0·014	0·220	0·065	0·011	0·173
Succinic acid	0·334	0·042	0·445	0·297	0·037	0·395
Alcohol	0·245	0·061	0·163	0·238	0·059	0·158
Total	0·741	0·131	1·043	0·761	0·115	1·152
Calculated for glucose	0·766	0·127	1·022	0·811	0·135	1·080
Calculated on basis of the carbon	0·741	0·124	0·988	0·761	0·127	1·013

Under anaerobic conditions greater variations occur in the proportion of alcohol to acetic acid than under aerobic conditions, and it would appear that one of the effects of the introduction of oxygen during the fermentation is to inhibit the mechanism of auto-reduction, which is responsible for the variations in alcohol when such occur.

Contrary to expectation, the products of aerobic fermentation contain not more, but less, oxygen than the corresponding products of anaerobic fermentation of glucose; but there is a gain of oxygen in both cases upon the original glucose. If, as seems likely, this extra oxygen comes from the water, then it would appear that one of the effects of the introduction of oxygen is to diminish the part played by water in the reactions.

In conclusion we wish to express our thanks to Prof. F. Gowland Hopkins, in whose laboratory this work was done.

Anthocyanins and Anthocyanidins. Part IV.—*Observations on:* (a) *Anthocyan Colours in Flowers, and* (b) *the Formation of Anthocyans in Plants.*

By Arthur Ernest Everest, D.Sc., Ph.D., F.I.C., and Archibald John Hall, B.Sc., A.I.C.

(Communicated by Prof. F. Keeble, F.R.S.—Received February 2, 1921.)

In a recent paper, Shibata, Shibata and Kasiwagi,* dealing with the question of the colours observed in flowers, and the condition of the anthocyan pigments as they exist in plants, arrived at conclusions that differ considerably from those of Willstätter and of Everest. In view of this, and of the fact that Shibata, Shibata and Kasiwagi's paper has passed into reviews, the present authors feel that the publication of some observations that they have made, both before the publication of the paper referred to, and since receiving it, as confirmation of the present authors' results and extension of the field, will not be out of place.

Results of experiments upon the formation of anthocyan pigments are also described, and important conclusions drawn from them.

The Constitution of the Blue Anthocyan Pigments in Flowers.

Willstätter and Everest† as the result of their examination of the pigments of the cornflower and of preliminary investigations upon other flowers, concluded that the blue colour in the cornflower was due to an alkali, or alkali-earth, salt of a phenolic substance which was violet in the free state, and which was also capable of forming red oxonium salts with acids. This simple explanation of the main colour changes being due to changes in the condition of the cell sap in the plants concerned, has been elaborated by Willstätter and Mallison‡ to show how such a supposition, coupled with the effects obtained by the presence of other plant colouring matters (flavonols, carrotinoids, etc.) and of varying concentration of colour in the cell sap, is capable of explaining all the lovely variations seen in plant life.

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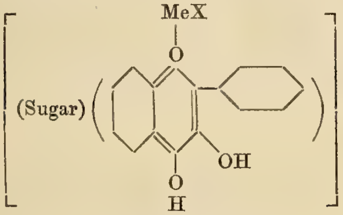
* 'J. Amer. Chem. Soc.,' vol. 41, p. 208 (1919).

† 'Ann.,' vol. 401, p. 189 (1913).

‡ 'Ann.,' vol. 408, p. 147 (1915).

Shibata, Shibata and Kasiwagi challenge the evidence of the existence of the anthocyan pigments in the three forms suggested by Willstätter and Everest, and to replace the conclusions of Willstätter and Everest, and Willstätter and Mallison, Shibata, Shibata and Kasiwagi put forward the following as an explanation of the various flower colours:—

(1) "The metal organic or complex compounds of reduced flavonal glucosides  $[MeX_2]_n$ are the most important factor in the production of flower colours."

(2) "The 'blue' anthocyanins are the complex compounds of reduced flavonol glucosides, which possess several hydroxyl groups belonging to the flavonol nucleus besides those of sugar molecules, and the metal with which they are coordinated is probably calcium or magnesium, for the salts of these metals are always present in the plant cells."

(3) "The 'violet,' 'violescent red' or 'red,' pigments are either the analogous metallic complex compounds of flavonol glucosides, which contain fewer of the auxochromic hydroxyl groups, or a mixture of the blue pigments and their decomposition products by excess of acids, *i.e.*, the red oxonium salts of R. Willstätter."

They then add, "Our conception of the colour variation of the anthocyans is, we believe, free from the objections raised in the preceding pages, and moreover, the blue pigments are accessible, while the phenolates and inner salts are wanting an experimental support."

Before passing to observations concerning these new suggestions, it is advantageous to consider carefully the experimental evidence that is available in support of them, as compared with that upon which the earlier conclusions of Willstätter and Everest were founded and developed.

Willstätter and Everest's conclusions are supported by considerably more experimental evidence than that referred to by Shibata, Shibata and Kasiwagi, and it would appear that these authors have overlooked some of the later work of Willstätter and his collaborators.

Willstätter and Everest (*loc. cit.*) produced evidence that proved the formation of oxonium salts in red anthocyanins, and made it highly probable that the blue pigment of the cornflower was the potassium or calcium salt of cyanin. Willstätter and Mieg proved that the violet flowers of wild larkspur contained the neutral colour base of delphinin,* whilst Willstätter and Bolton† showed that in the scarlet pelargonium the pigment pelargonin was accompanied by a considerable amount of tartaric acid, and that it could form stable salts (oxonium) with such weak acids as acetic acid. The further work of Willstätter and his collaborators has resulted in the isolation of the colour bases of the pigments: pelargonin, cyanidin and delphinin, and also of the colourless pseudo-bases of pelargonidin, cyanidin and delphinidin.

In support of their theory of complex salts, and their explanation of the colours in flowers based thereon, Shibata, Shibata and Kasiwagi describe the preparation of various blue and blue-green compounds and give analyses of them.

Exhaustive and careful examination of the data given by them has brought the present authors to the conclusion that much of the evidence they set out is of an unsatisfactory character, and that their conclusions concerning their experimental results are misleading.

The present authors are convinced that very valuable results may often be obtained by the careful examination of plant extracts or impure pigments, but only if and when due allowance is made for the fact that impurities are present and for the nature of the impurities. In dealing with plant pigments, such allowances can only be made when the investigator concerned has himself made a careful study of the pure pigments and has considerable first-hand knowledge of these bodies. It is often the case that access to even the smallest amount of a pure pigment will save endless incorrect deduction from experimental results. It appears to the present authors that sufficient weight has not been attached to this by Shibata, Shibata and Kasiwagi.

Not only is this the case, but in places their arguments are inconsistent with statements made in other parts of their paper.

Willstätter, and also Everest, have found, as the result of quantitative work, that under conditions such as Shibata, Shibata and Kasiwagi described for the isolation of their green and blue pigments, the flavonol used is never quantitatively converted into the corresponding anthocyan. Furthermore, it is well known that the addition of metallic acetates in alcoholic solution to flavonol derivatives such as myricetin gives rise to the formation of metal phenolic salts of the flavonols. Hence, when they precipitated their green pigments, it follows that they obtained, not as they imagined, an anthocyan salt, but a mixture of magnesium salts of the anthocyan and flavonol. Not only so, but magnesium acetate is appreciably soluble in alcohol, and is precipitated from its solution by means of ether. Their extraction of the precipitate with ether—to remove myricetin—would be of no avail, as the myricetin would be present, not free, but combined with the metal. Exactly similar results have been obtained by the present authors by mixing a trace of anthocyan with a flavonol in alcoholic solution, adding magnesium acetate, and precipitating and washing with ether. The green and greenish-blue pigments of Shibata, Shibata and Kasiwagi can be matched in this way with ease; they do not represent pure compounds, and Shibata, Shibata and Kasiwagi's analytical figures are of no value.

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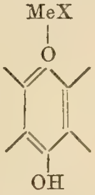
* 'Ann.,' vol. 408, p. 67 (1915).

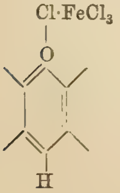
† 'Ann.,' vol. 408, p. 53 (1915).

An important case of inconsistency arises in connection with the statements concerning their complex compounds. They argue that the blue anthocyan colours are complex metallic salts, and describe, as mentioned above, the preparation of what they consider to be these complex salts, but, on p. 211, they state that the products which they prepared "are soluble in water as well as in alcohol," and, on pp. 217, 218, say of their green pigments that they are soluble in alcohol as well as water. Despite this, they say on p. 213 that the "sparing solubility of many blue or bluish-violet anthocyanins in alcohol is quite consistent with the magnitude of the complex molecules." This latter statement would indicate that their compounds were not the same as the natural pigments.

The brownish red crystalline powder obtained by them (p. 218) was undoubtedly a mixture of flavonol and anthocyan, for the green or blue substances from which it was prepared must have contained the magnesium salt of myricetin.

It should further be noted that they formulate their complex salts as containing a central group of the type: 

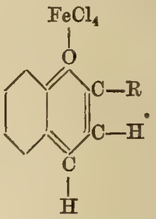
whereas there is very considerable chemical evidence (see below) that at least in the case of the salts with ferric chloride, the pyrylium ring is correctly represented thus:



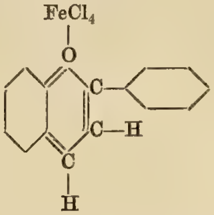
Comparison of the Properties of various Salts and simple Phenolates of Anthocyans and related Pyrylium Derivatives.

From the authors' own observations and from previous work of other investigators, it is now apparent that two distinct classes of metallic salts of anthocyan pigments (or related hydroxylated pyrylium compounds) exist.

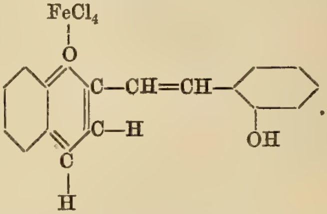
The work of others has shown that in numerous pyrylium compounds (oxonium salts) the addition of ferric chloride gives rise to iron compounds which can be represented by simple formulæ of the type:



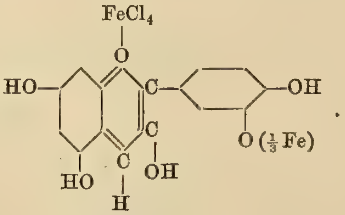
They appear to be additive compounds of the iron salt and the oxonium salt of the pyrylium derivative. In connection with other work, the present authors have isolated compounds in which the iron chloride-content agrees with the structures:



and



When dilute solutions of the oxonium salts of many anthocyans have small amounts of iron salts added to them, characteristic intensely coloured solutions result (blue in the case of ferric chloride to cyanin or violanin chloride) which are stable and show no tendency to decolorise on standing. The present authors agree that these are complex salts, but consider they are probably of the type:



On the other hand, sodium, potassium, calcium, or magnesium salts of the anthocyans, when in solutions of similar dilution, gradually decolorise on standing. The present authors are of the opinion that these are simple phenolates, and related to the alkali salts of the flavonols described by A. G. Perkin and others.

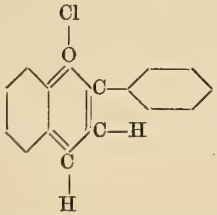
This indicates clearly a very marked line of differentiation between the action of iron salts and those of the alkali and alkali-earth metals on anthocyan pigments.

Confirmation and additional information concerning this difference has now been obtained by adding a trace of the salts of various metals to decolorised solutions of violanin or cyanin chlorine. In the case of iron, copper, and tin, although no immediate colour change occurred, a full intense blue gradually developed and was stable, whereas in the case of the salts of sodium, calcium, and magnesium, even on long standing, no return of colour at all occurred. (The presence of the anthocyan in the colourless form was proved in each case after the experiment by the addition of acid, whereby the oxonium salt was regenerated in full.)

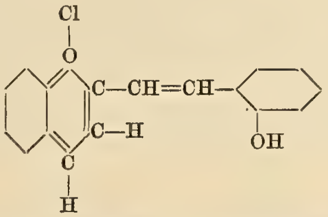
In connexion with the complex iron salts, Willstätter's observations concerning the methylation of delphinidin upon the colour changes produced when the oxonium salts of delphinidin, or its various methyl ethers, are treated with iron chloride have made it abundantly clear that the final change from purple (or violet) to blue is, in these instances, dependent upon the reactivity of certain phenolic hydroxy groups.

The present authors have found that when iron chloride is added to the solutions of the compounds

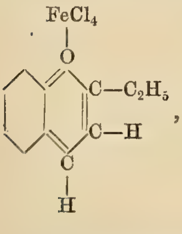
(I)



and (II)



there is no colour change beyond slight intensification, although definite iron compounds are formed, and the tendency to decolorise in dilute solution is very greatly reduced. This absence of marked colour change on passing from the oxonium chloride to the iron double salts has also been recorded in a number of cases, including the following:—



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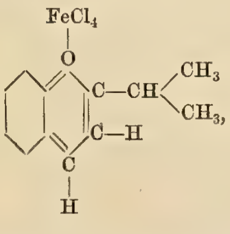

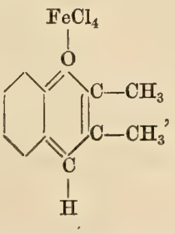

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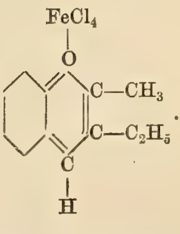

.*

On the other hand, the present authors have observed that, when a trace of the acetates of an alkali or alkaline-earth metal is added to an alcoholic solution of (I) or (II), the colour is immediately discharged, due to the pseudo-base formation. This clearly indicates that the acetates of these metals have not the power of forming stable addition complexes with the oxonium group. In the absence of any phenolic hydroxyls, no second colour change takes place. In contrast to the above, when sodium acetate is added to an alcoholic solution of cyanin chloride, the first colour change (red to purple) due to colour base formation, is followed by a second colour change (purple to blue) due to phenolic salt formation. The blue phenolic salt thus produced decolorises when allowed to stand in dilute solution.

---

* *Cf.* Decker and Fellenberg, 'Ann.,' vol. 364, p. 1 (1909).

The above observations have brought the authors to the conclusion that, in the fine blue complex iron salts of anthocyans, the blue colour itself is due to an interaction of the metal with a phenolic group (or groups), whilst the stability of the colour in dilute solution is due to the attachment of the metallic salt to the oxonium complex. In the case of the blue alkali, or alkaline-earth salts of anthocyans, the blue colour is due to a phenolic grouping, whilst the lack of stability in dilute solution is due to the inability of these salts to form stable complexes with the oxonium group.

The authors consider that it is most probable that all the anthocyan pigments form additive salts with ferric chloride, in which the iron salt is attached to the oxonium group, but that characteristic colours are only produced when suitably placed phenolic OH groups also react.

Conclusions concerning the Constitution of Blue Anthocyan Pigments in Flowers.

From the above it will be seen that the blue colours of anthocyan-containing flowers may be due to the presence of either—

(i) Anthocyan phenolates of alkali or alkaline-earth metals; or

(ii) Complex anthocyan-iron salts. (It is unlikely that copper or tin will take part.)

In deciding under which of these heads we must classify the actual blue pigments occurring in flowers, observations as to whether or no dilute solutions of their colouring matters become decolorised on standing should serve as a reliable guide.

In the case of at least three blue flowers (cornflower, iris, and violet), there is definite recorded evidence* that solutions of the blue pigments present in each of these flowers decolorise on standing. As there is also record that the decolorisation in each case is due to pseudo-base formation, and not to decomposition, the conclusion must be drawn that no complex

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* Fremy and Cloez, 'J. f. pr. Chem.,' vol. 62, p. 269 (1854); Willstätter and Everest 'Ann.,' vol. 401, p. 189 (1913).

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anthocyan-iron salt is present in these flowers. The blue colour must therefore be due to the presence of anthocyan phenolates of the alkali or alkaline-earth metals.

In the case of the flowers of the hydrangea, in which the pigment assumes a blue colour after the addition of iron salts to the soil in which the plant is grown, it is quite possible that we have an example of the other type, and that the pigment is a complex anthocyan-iron salt. As the authors have not yet been able to examine these flowers, a decision upon this point must be left until later.

The Mode of Formation of Anthocyan Pigments in Plants.

The extended botanical investigations of Keeble, Armstrong and Jones,* and of Wheldale,† indicated that a relationship existed between the yellow sap-pigments and the anthocyans, whilst Everest has described work which brought him to the conclusion that in Nature the anthocyan colouring matters are produced from yellow sap pigments (of the flavonol group) by reduction.‡

The present authors now describe observations which appear to prove that, at least in some cases, this conclusion is fully justified. The experiments now described were undertaken with a view to extending our insight into the mechanism of production of the anthocyan pigments in Nature.

Although it has already been established by the work of Willstätter and of Everest, that the anthocyan pigments may be produced by reduction of flavonol derivatives, it has not previously been so clearly indicated whether, in Nature, flavonols are first formed and from them the anthocyans, or the anthocyans directly synthesised and the flavonols produced from them by oxidation.

Additional interest was given to the matter by a lecture by Dr. R. Robinson, F.R.S. (then Professor of Chemistry in the University of Liverpool), at the University of Manchester, in which he dealt with the theoretically possible synthesis of plant products from sugars. He indicated the manner in which the synthesis of anthocyans from sugars *might* take place in Nature, and also pointed out that it was less easy to indicate how flavonols could be synthesised from sugars. As a result of these theoretical considerations, Dr. Robinson contended that the flavonols were formed from anthocyans by oxidation, the anthocyans being the primary synthetic products.

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* 'Roy. Soc. Proc.,' B, vol. 85, p. 214 (1912); vol. 86, pp. 308 and 318 (1913); vol. 87, p. 113 (1913); and Keeble and Armstrong, 'Jour. Genetics,' vol. 2, p. 277 (1913).

† 'Camb. Phil. Soc. Proc.,' vol. 15, p. 137 (1909); 'Jour. Genetics,' vol. 1, p. 133 (1911); 'Biochem. Jour.,' vol. 7, p. 87 (1913).

‡ 'Roy. Soc. Proc.,' B, vol. 87, p. 444; vol. 88, p. 326; and vol. 90, p. 251.

The present paper describes an attempt made by the authors to indicate which of these possible processes of synthesis is in reality adopted by Nature.

If Robinson's suggestions are correct, then in those flowers which contain anthocyan pigments, it would be anticipated that the colour of the anthocyan would develop before any trace of flavonol could be detected. No published observations are yet available to support this, and the present results indicate that the opposite is the case. Further, even pale yellow, or white, flowers which contain flavonol derivatives but no anthocyans, would be expected to pass through a stage in which they were coloured by the anthocyans from which the flavonols were to be formed; unless it is assumed that the transformation from anthocyan to flavonol, in such cases, is always complete, and proceeds more rapidly than the formation of anthocyan.

If on the other hand flavonols are first synthesized in Nature and anthocyans formed from them only in those cases where the conditions for reduction are available, then the stages would be simplified. In the pale yellow or white petals containing flavonols there would be gradual development without marked colour changes. This is what occurs. In the flowers which are coloured by anthocyans it may well be expected that at least in most cases the very small buds will be pale yellow, or colourless, and that a certain amount of flavonol will be developed before the anthocyan is formed and becomes obvious by its tints. The present investigation proves that this is the case. Moreover, it is likely that flavonols would be found alongside anthocyans in flowers containing the latter. Published observations agree with this.

The authors consider that the present results strengthen the evidence that the anthocyan pigments are formed from flavonols. Only by assumptions such as that indicated above, could the reverse be reconciled with the experimental evidence at present available.

Experimental.

As myricetin was not available to the authors, and Shibata, Shibata and Kasiwagi state in their paper that the reduction of quercetin or of quercitrin proceeds "just the same as myricetin or myricitrin," the authors carried out a reduction of quercitrin under exactly the conditions given in the paper of the above mentioned authors. The result was that a green solution having bright green fluorescence was obtained from which a green pigment (mixture of pigments) could be precipitated by means of ether. The green precipitate remained green on washing with ether. An exactly similar result was obtained if the reduction was carried out by means of a very small amount of hydrochloric acid, using excess of magnesium, and when the reduction was completed, adding magnesium acetate.

In order to show that the pigments obtained by the above-mentioned authors were not pure products, experiments were then made as follows. Chemically pure crystalline samples of cyanin chloride, violanin chloride, and quercitrin were used.

(i) Magnesium acetate was added to a fresh alcoholic solution of (*a*) cyanin chloride; (*b*) violanin chloride. In each case a fine pure blue pigment was formed, which did not change when precipitated and washed with ether.

(ii) Magnesium acetate was added to a solution of quercitrin in alcohol; a bright yellow colour developed and yellow-green fluorescence. Ether precipitated a yellow pigment (Mg salt), which did not change when washed with ether.

(iii) Magnesium acetate was added to solutions containing (*a*) a mixture of cyanin chloride and quercitrin, and (*b*) a mixture of violanin chloride and quercitrin. In each case green pigments were at once produced, and the solutions showed the same bright green fluorescence as when the reduction above described was carried out. Precipitation and washing with ether left the green pigments (mixtures) unchanged.

(iv) An alcoholic solution of magnesium acetate was made, filtered, and ether added;—magnesium acetate was precipitated.

These results prove that the analyses of the green pigments described by the above mentioned authors are valueless.

By adjustment of the proportions of cyanin chloride and quercitrin in Experiment iii (*a*), the green pigment obtained by reduction of quercitrin was readily matched.

Further observations respecting the reaction of anthocyan pigments with metallic salts, which have been made from time to time, appear to be of sufficient interest to insert here. In particular, those on violanin, as this would most nearly correspond to the reduction products obtained from myricetin or myricitrin.

(i) To separate portions of a fresh solution of violanin chloride in alcohol (*circa* 95 per cent.) there were added: (*a*) magnesium acetate, (*b*) calcium acetate, (*c*) anhydrous sodium acetate; in each case a fine pure blue was at once produced, and on standing, the whole of the colour was gradually deposited as an indigo-blue flocculent precipitate. The colour of the blue solutions could not be distinguished from one another. The blue precipitates were all soluble in water, but were insoluble in absolute or 95 per cent. alcohol. The dilute solutions of these precipitates become decolorised on standing, owing to pseudo-base formation.

(ii) Effect of metallic salts on aqueous solutions of violanin chloride:—

(*a*) Addition of magnesium acetate gave a fine blue (with violanin mixed with quercitrin, green).

(*b*) Addition of ferrous sulphate gave a fine blue (with violanin mixed with quercitrin, green).

(*c*) Addition of copper sulphate produced a fine purple-blue, which passed to pure blue on warming, returning to purple-blue on cooling.

(iii) Cyanide chloride when in alcoholic solution behaved exactly like violanin chloride when magnesium acetate, anhydrous sodium acetate, or potassium acetate was added, a fine blue colour, followed by deposition of the blue pigment, being the result in each case.

(iv) Further observations that have been made by the present authors, and which have direct bearing upon the paper under discussion, are those upon the effect of adding various metallic salts to solutions of the colourless form of violanin. A solution was prepared by dissolving crystalline violanin chloride in a little alcohol, pouring this into water, and allowing to stand until decolorised. Separate portions of the solution were taken and salts added, with the results given below. (In each case one drop of a dilute solution of the salt was used.)

(*a*) Ferrous Ammonium Sulphate: no immediate colour, but fine blue gradually appeared.

(*b*) $CuSO_4$: no immediate colour, but fine blue gradually appeared.

(*c*) $SnCl_2$: no immediate colour, but fine blue gradually appeared.

(*d*) $FeCl_3$: great difficulty in obtaining colour as oxidation with destruction of pigment occurred; if very dilute solution of $FeCl_3$ was used, got fair but pale blue.

(*e*) $CaCl_2$: at once very pale green but colour did not increase.

(*f*) Magnesium Acetate: at once very pale green but colour did not increase.

(*g*) Lead acetate: at once very pale green but colour did not increase.

(*h*) Sodium Acetate: at once very pale green but colour did not increase.

(*i*) Na_2CO_3: rapidly yellow green; on standing, yellow and pigment destroyed.

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June 1, 1921.

NOTICE TO AUTHORS AND COMMUNICATORS.

The Council have had under consideration the rapid increase of the Society's expenditure on publications. In view of the necessity for economy, authors of papers are urgently requested to see that their communications are put in as concise a form as possible. Delay in decisions regarding publication, as well as subsequent trouble to authors, is often caused by diffuseness or prolixity. MSS. must be type-written or at least written in a legible hand, and properly prepared as copy for press. Type-written transcript should in all cases be carefully revised by the author before being presented. It is desirable that authors should retain copies of their MSS. for reference.

Every paper must be accompanied by a summary not exceeding 300 words in length.

Authors are requested to refer to a Memorandum on Mathematical Notation published in these 'Proceedings,' 1909, Series A, vol. 82, p. 14, and to adhere to the suggestions therein contained, so far as possible.

Authors are further requested to send in all drawings, diagrams or other illustrations in a state suitable for direct photographic reproduction. They should be drawn on a large scale in Indian ink on a smooth white surface, with temporary lettering in pencil. Great care should be exercised in selecting only those that are essential. Where the illustrations are numerous, much time would be saved if the authors would indicate in advance those which, if a reduction of their number is found to be required, might be omitted with least inconvenience.

"It shall be the duty of each Fellow or Foreign Member to satisfy himself that any letter, report or other paper which he may communicate, is suitable to be read before the Society."—Statute 64, Cap. xii.

NOTICES TO FELLOWS OF THE ROYAL SOCIETY.

The Council have directed that the Minutes of the Meetings of the Society shall be sent out as an inset in the 'Proceedings,' separately paged. In future they will not be republished in the 'Year-Book.'

The 'Proceedings' are sent in the ordinary course by post to those Fellows of the Society who have applied for them. On application to Messrs. Harrison and Sons, Ltd., St. Martin's Lane, London, W.C. 2, these will be bound in volumes, in cloth, for 5s., or the cases for binding may be purchased, price 2s. 3d.

The 'Philosophical Transactions' are now issued in the form of separate Papers, and are delivered post free, immediately on publication, to those Fellows who desire to have them in that form. On application to Messrs. Harrison and Sons, Ltd., St. Martin's Lane, London, W.C. 2, these issues will be retained, and supplied in volume form in cloth binding.

SUBSCRIPTION TO THE PROCEEDINGS OF THE ROYAL SOCIETY.

The 'Proceedings' are on sale to the public, on publication, at the prices appearing on the wrappers; or may be obtained by subscription, paid in advance.

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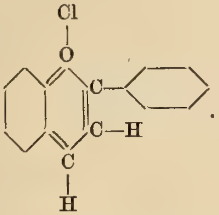
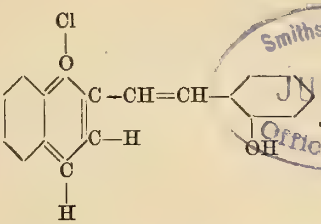
Observations concerning Related Pyrylium Compounds.

In connection with other work, the present authors have prepared the mother substance from which all anthocyans are derived (1), and also the related compound (2):—

(1)


(2)


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The compound (1) has been prepared as the oxonium chloride, as a double salt with ferric chloride, and as the free base, whilst compound (2) has been isolated as oxonium chloride, and as a double salt with ferric chloride.

Observations on the Colour of Buds and Young Flowers.

A considerable number of plants, including auricula, apple, azalea, polyanthus (red), primrose (red), violas, etc., were selected whose flowers had well-marked anthocyan coloured petals when fully developed. Buds were taken in each case from the same plant at intervals and examined, until the fully developed flower had been reached. In some cases the anthocyan did not develop until light fell on the petals, in others the anthocyan (even quite deep shades) developed freely before the bud was unfolded. Despite this, in nearly every case it was evident that, before the anthocyan colour appeared, there was a stage in which the petals were yellow or colourless, and contained dissolved substances which turned yellow on exposure to ammonia. In the cases of some very deep coloured flowers, *e.g.*, black violas, the buds were highly coloured, even when extremely small.

A number of flowers which when fully developed contain only flavonols were likewise examined, including primrose (pale yellow), roses (various), chrysanthemum, etc., but in no case could any intermediate anthocyan formation be observed.

Attempts to Extract Flavonols from Buds (before Anthocyans are present) and Produce Anthocyan Colorations from them.

Whilst the above observations showed that it was probable that flavonols were formed before anthocyans, the authors desired to obtain further proof of the presence of the flavonols. To this end they chose buds of a red rose, and of a medium coloured mauve viola (Maggie Mott), as being suitable on account of the fact that they attained a fair size before any sign of anthocyan formation became observable. Buds of these were gathered of the largest size attainable before any sign of anthocyan formation had appeared. The young petals were well crushed in warm alcohol (*circa* 95 per cent.), and, after standing for a short time, the extract was filtered, and the filtrate acidified by the addition of a small amount of concentrated hydrochloric acid. When the acid was added, the filtrate changed slightly from pale yellow to colourless (on the other hand, ammonia gave a deeper yellow), but in neither case was any red tint due to anthocyan observed. The acidified filtrate was divided into two portions; to one a little magnesium was added, the second was retained as a check. In the case of both rose and viola, a gradual change to a clear pale red took place in the colour of the liquid to which the magnesium had been added. The portion of the acidified extract which had not had magnesium added to it remained colourless. These observations leave no doubt that, in the cases examined, there were flavonol derivatives present in the buds which would later have developed anthocyan colours, but in which up to that time no anthocyan had been formed.

The authors intend to extend these observations.

A Remarkable Flint Implement from Selsey Bill.

By Sir Ray Lankester, K.C.B., F.R.S.

(Received January 18, 1921.)

[Plates 8–11.]

It is desirable that the large rostrate flint implement drawn in figs. 1, 2, and 3, and the hammer-stones drawn in figs. 4 and 5 should be made known without further delay to students of prehistoric archæology. These specimens were placed at my disposal in 1912 by Mr. Edward Heron Allen, F.R.S., and have now been presented to the Department of Ethnology and Mediæval Antiquities of the British Museum. They were briefly mentioned by me in a postscript to my paper on "The Discovery of a novel type of Flint Implements below the base of the Red Crag of Suffolk."*

These specimens were (as I am informed by Mr. Heron Allen) found in November, 1911, when the shingle was suddenly washed away below the alluvial cliffs and Raised Beach on the west shore of Selsey Bill, exposing a band of yellow clay, about 100 yards long and 4 yards in breadth. Lying on this were large flints, and the first which attracted Mr. Heron Allen's attention were those described and figured in this communication. Besides these he collected a barrowful of large broken flints, which he later submitted to my examination. The shape of many was suggestive of preliminary trimming, with a view to chipping as "pointed" implements, but the big rostrate implement and the two hammer-stones here figured, which were in the first instance secured by Mr. Heron Allen, were the only flints among the lot which had been obviously and certainly shaped by man. Subsequently many other large fractured flints—some of two or three pounds in weight—were found accumulated in patches on the sand at low tide, 200 yards further in a south-westerly direction. Though the shape of many of these (which were collected by visitors to the spot) was suggestive, none were discovered the fracturing of which could, at that time, be decisively attributed to human agency. After examining some hundred or more selected by Mr. Heron Allen and other observers, I came to that conclusion. It is, however, of course to be expected that other humanly-worked flints besides the three specimens now described and figured will eventually come to light at this spot.*

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* 'Phil. Trans.,' B, vol. 202 p. 332.

The clay on which the flints collected by Mr. Heron Allen were resting is of Bracklesham age. It is overlaid in the low cliff exposed at Selsey by a light-coloured gravel, which has been described by the late Mr. Clement Reid as Coombe rock.

The large "worked" flint, the position and finding of which are described above, is represented in figs. 1, 2, and 3. It weighs 5 lb. 12 oz., and is 8 inches long and 5½ inches broad. It has been shaped by numerous—mostly large and coarse—fractures to the form of a rostro-carinate implement, having a well-marked tapering "rostrum" (fig. 1) and a relatively flat ventral area (fig. 2), corresponding to the ventral plane of a typical "rostro-carinate." But there is no "carina"; that is to say, the essential shaping has not been attained by the removal of large flakes of flint right and left of the mid-dorsal line, so as to leave a median *arête* or carina. On the contrary, the shaping of the rostral region has been attained by the removal of one very large flake on the left side (fig. 1, *A*) and by a series of four parallel ribbon-like fractures of the upper surface (fig. 1, *1*, *2*, and fig. 2, *2*, *3*, *4*). Adjoining these on the right side, is the scar (fig. 2, 5) of a broader fifth flake, truncated by a sixth (see fig. 2, *6*).

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* [*Note added March* 8, 1921.—Some of the large angular flints from Selsey would re-pay further examination. Mr. Edward Heron Allen has recently sent me one which, I agree with him, must be admitted as bearing conclusive evidence of flaking by human agency.]

The rest of the entire surface of the specimen has been trimmed by very numerous blows, some of great power. These have removed all of the original cortex of the flint nodule operated upon, excepting a few small patches, marked *cort.* in figs. 1, 2, and 3. The trimming fractures of the general surface have produced a few "rippled-marked" scars (fig. 2, *R*, *S*, *T*, *V*, *X*). For the most part, the surface presents a series of irregular convexities and concavities of a silky black appearance.

The specimen is not iron-stained, and is entirely free from water-wear or water-polish—though the fracture-edges are not sharp, but feel smooth and blunted when the finger is passed over them.

I have already mentioned the existence of a few small patches of the original "cortex" or "bark" of the nodule. There is here and there—on the ventral surface—(for instance, in the region about S in fig. 2)—a superficial change of the black colour of the fractured flint surface to grey. This decomposition of black flint usually, but not always, takes place on a broken surface—commencing as a pale bluish "bloom." It becomes whiter and more opaque, with longer duration of exposure. It is convenient to call this change "lactescence," as I have proposed in 'Proc. Roy. Soc.,' 1920.

Lactescence is seen commencing as a very slight "blueing" of the fractured surface in large blocks of flint at Brandon—which have been broken and exposed in the knappers' yards for as short a time as six months. On the other hand, fractured flints are common enough with black surface showing no trace of lactescence, although fractured many thousands of years ago. The conditions which favour and those which prevent this change of surface have never been ascertained.

I propose to call this implement the "Selsey rostrate." If others of the same form should hereafter be found at Selsey, they may be named Selsey rostrate B, C, D, and so on.

The Selsey rostrate was not manufactured from a "blank" trimmed to a tabular shape nor from a block of natural tabular flint. It is very usual for palæolithic implements of smaller size than this to have been flaked into shape from a piece of flint measuring about 5 inches in length, 3 inches in breadth, and 2 inches in thickness. The tabular form was either produced by the preliminary "roughing out" of a nodule or, more rarely, owed its initial shape to the fact that the piece selected for use was a block of *natural* tabular flint.

The Selsey rostrate is an unusually big and heavy implement, and was chipped out of a big heavy nodule, measuring probably not less than 9 inches in length, 6 inches in breadth, and 4 inches in thickness. It is too heavy for use, as a pick or axe, in one hand, and is not shaped for being held by both hands. The inference from its great size, and from the powerful blows which have been used, with skilful precision, in its manufacture, is that it was made and used by a man of larger hand and limb than are possessed by any modern race. It would be a mistake to attach much importance to this suggestion, since we are singularly devoid of any well-founded opinions as to the way in which the larger palæolithic flint implements known to us were brought into effective use, and the purpose or purposes for which they were used.

In regard to flint implements of the largest size, it is to be noted that we know of rare specimens of tongue-shaped or pick-like implements from river-terrace gravels of Acheulæan and Chellæan age, as much as 11½ inches in length and 5 lb. in weight. Such a specimen is in the Department of Ethnology of the British Museum, another in the National Museum of Antiquities of St. Germain, and one in the collection of Mr. Scott, of Bournemouth. Possibly these unusually large implements were not for ordinary use, but were ceremonial emblems of authority.* Very large neolithic adzes ("celts") of stone (not flint) are found in the north of Scotland and Ireland. One as much as 10 inches in length, and weighing 5 lb. 4 oz., is in the Sturge collection at the British Museum. Undoubtedly, these were mounted on wooden handles, in a fashion similar to that employed by modern stone-weaponed races for similar large adzes of polished stone. The palæolithic flint implements from our river-terrace gravels—of the more frequent size and shape—namely, ovate, 4½ inches long and 3 inches broad, weighing ¾ lb.—and tongue-shaped specimens—often 9 inches long and 4 inches broad at the butt, weighing 1¾ lb.—are carefully shaped and well balanced for use in the hand. They could be readily used either with or without a "mount" or handle.

The question as to the mode of use of certain exceptionally large and heavy flint implements comparable to the Selsey rostrate, has a separate importance. I know of two only which are comparable in weight and size to the Selsey specimen, and these are from very ancient deposits held to be much anterior (in geological time) to the terrace gravels of our river valleys. The first of these is the large more or less cylindrical flint, flaked at one end to a cutting edge, which was discovered by Mr. Reid Moir, in 1909, in the Sub-crag detritus bed in Bolton and Laughlin's large clay pit at Whitton, Ipswich, and figured by me.* This flint is 10¼ inches in length and weighs 8 lb. 12 oz. It is too heavy for use in one hand but might well have been held by both hands and used for "pounding."

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* [*Note added March* 8, 1921.—A very large oval flint implement from the gravel at Taplow has recently been presented to the Natural History Museum by Mr. Treacher. It is of superior workmanship, perfectly symmetrical and clearly flaked. It weighs 6 lbs. 2 ozs., and is 12⅛ inches long. Apparently it is of Acheulæan age and is, I believe, the largest and most skilfully worked implement known from that horizon. It was probably a "ceremonial" emblem of authority and not intended for ordinary use.]

The second is the most remarkable among a great number of very large worked flints, recently discovered by Mr. Reid Moir below the forest-bed at Cromer in such a position as to indicate a workshop or flint-workers' "floor"—of an age anterior to that of our river-terrace gravels. Mr. Moir will have given a preliminary account of his discovery before the present communication is published. The largest of the worked flints from this newly-discovered "floor" weighs 7 lb. 6 oz., is 10 inches in length, 5½ inches broad and at the "butt" end is 4 inches thick. It has a rostrate form, a relatively flattened ventral surface and is richly worked all over by large coarse flaking of indubitable human origin. It presents a marked resemblance—both in general form and in the character of the flaking of its surface—to the Selsey rostrate as well as in its great size and weight.

The point to which I wish to draw attention in regard to these three unusually large and heavy flint implements, is that they belong to a very early period, antecedent to that of the familiar tongue-shaped and ovate implements of Chellæan and Acheulæan age. This is certainly true of both Mr. Reid Moir's big implements, and is probably true of the Selsey rostrate.

The early age of these big implements is consistent with the hypothesis that they were made and used by an early race of men of heavier build than that which succeeded them and produced the abundant ovates and tongue-shaped implements of our terrace gravels.

Whether made by an exceptionally big race or by men of the modern size, the use of heavy big flint implements, such as the three which I have here cited, presents a problem. If used merely as hammers or as club-heads they would be unwieldy and would not require any special shaping—such as would give precision to a smaller implement. The only suggestion I can offer as to their use besides that of "pounding" or breaking into the cavities of the bones of large animals in order to extract marrow, brain, etc.—is that they were employed either affixed to a handle or held by the two hands for the purpose of breaking a hole in the ice on the surface of a lake or marsh pool. Fish come to such openings in the ice and are then readily speared or captured.

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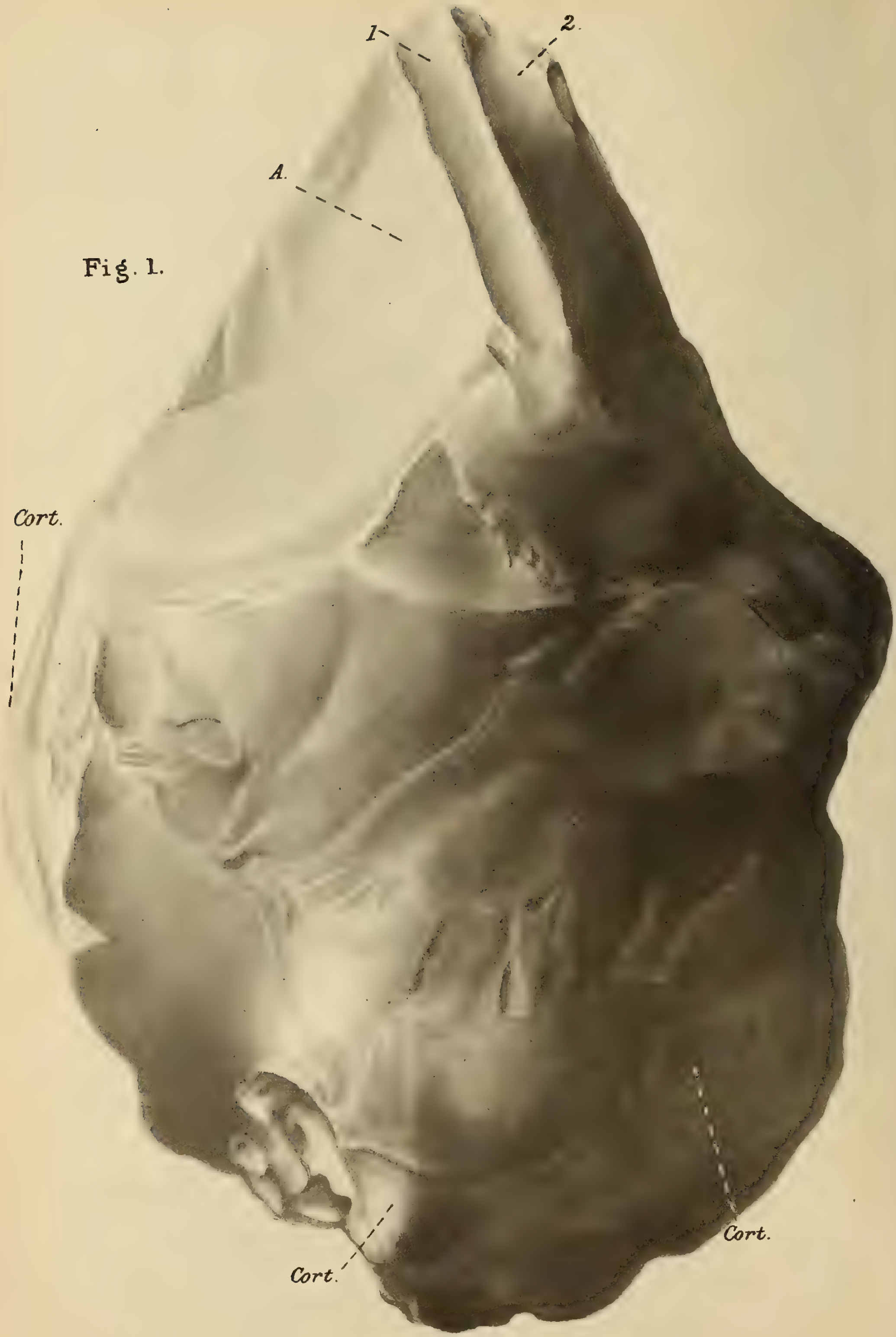
* 'Phil. Trans.,' B, vol. 202, pp. 323, 324 (1912).

Lankester, *Roy. Soc. Proc. B, Vol. 92, Pl. 8.*

Fig. 1.


1
2.
A.
Cort.
Cort.
Cort.

G.M.Woodward del.

Huth coll.

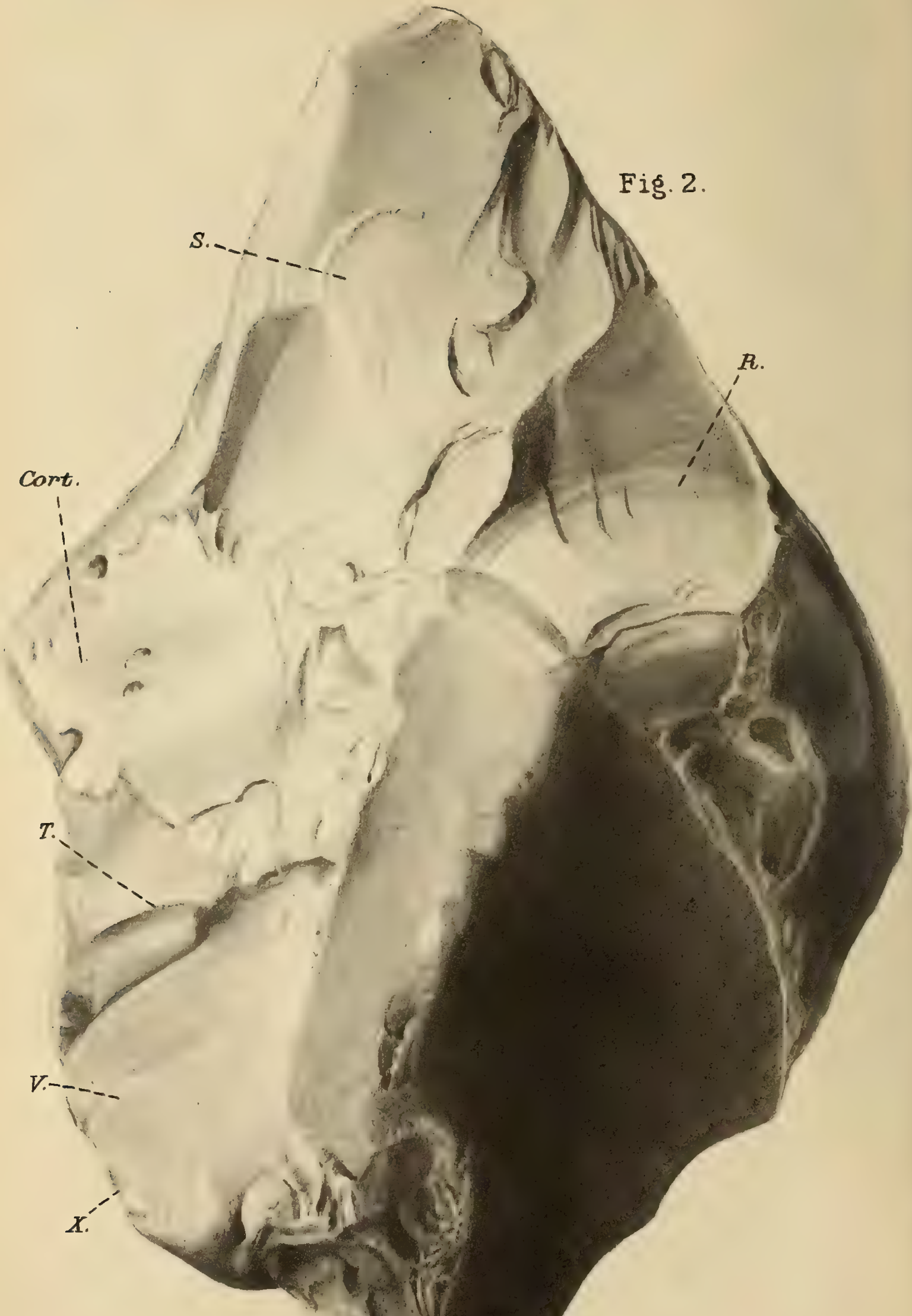


Fig. 2.

G.M.Woodward del.

Huth coll.

Huth coll.

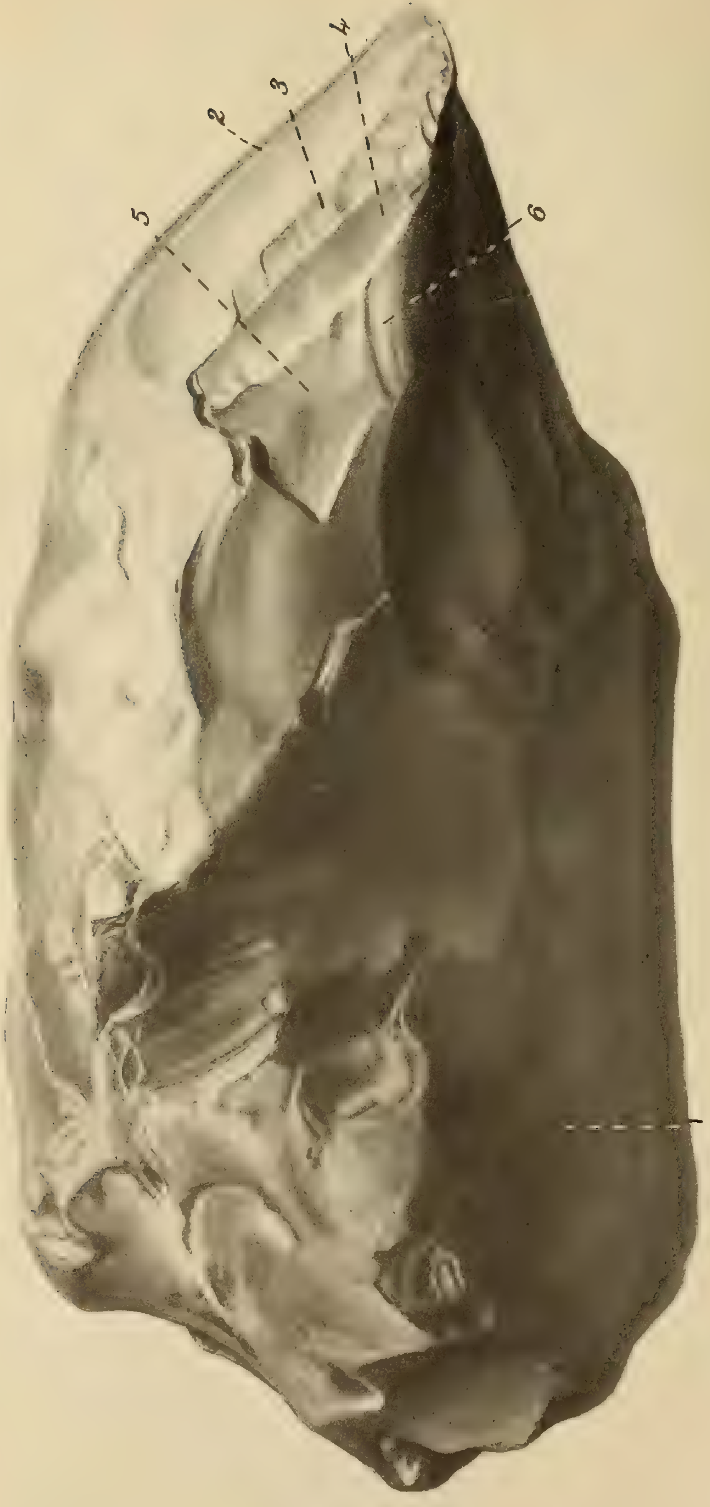


Fig. 3.

G. M. Woodward del.

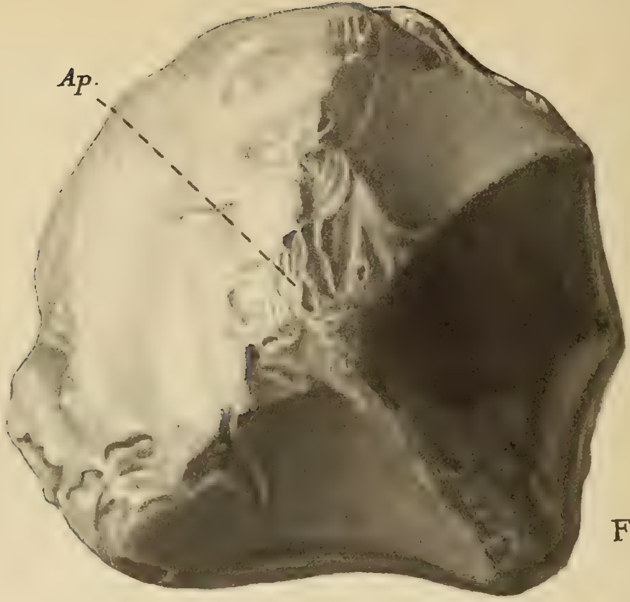


Fig. 5.

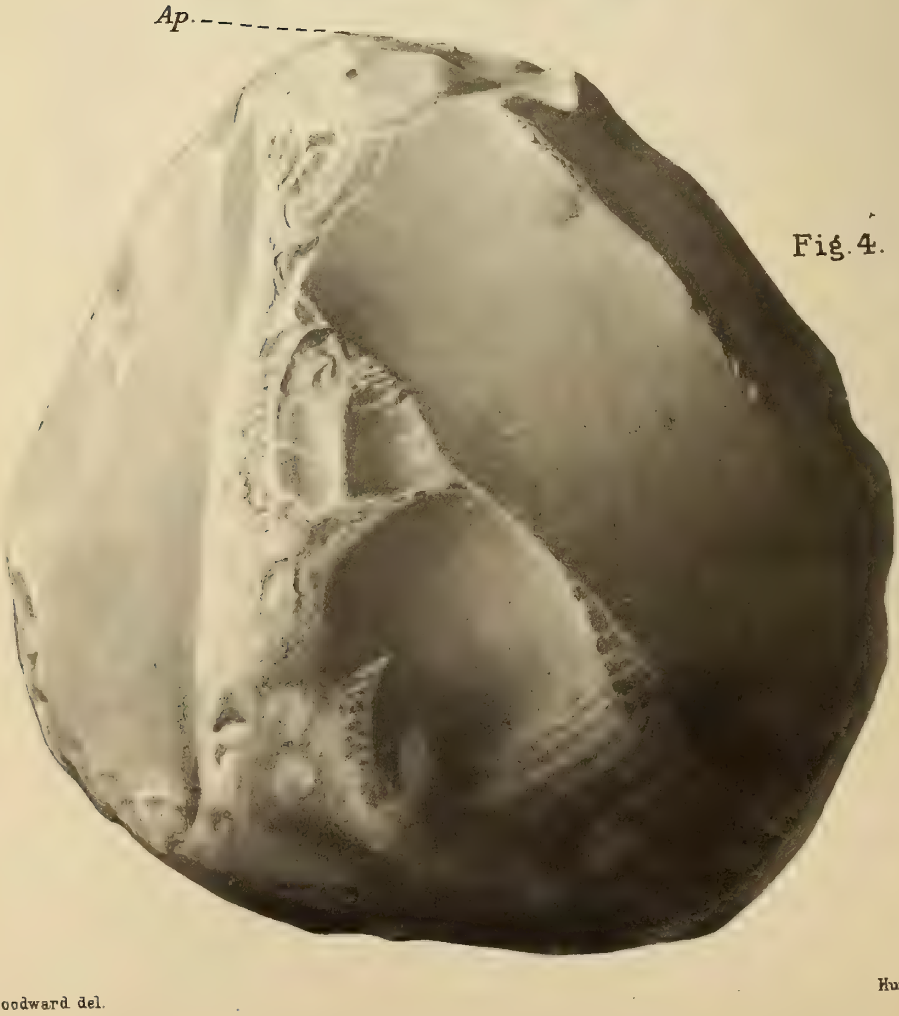


Fig. 4.

G. M. Woodward del.

Huth coll.

In figs. 4 and 5 careful drawings are given of two hammer-stones—a larger and a smaller—found in association with the Selsey rostrate and presenting the same colouring and mineral condition of the flint which characterise that implement. They have been flaked so as to furnish a prominent apex which shows evidence of its use as the striking surface in the fact that it is more or less comminuted. The smaller of the two hammer-stones is flaked so as to form a four-sided pyramid and is similar in this respect to the four-sided pyramidal hammer-stone from the Sub-crag detritus or nodule bed figured in my paper, 'Phil. Trans.,' B, vol. 202, p. 313. It is a fact of distinct importance tending to associate the specimens from Selsey Bill with those of the Sub-crag deposit of Suffolk, that a four-sided pyramidal hammer-stone is among the implements discovered in both localities.

EXPLANATION OF PLATES 8 TO 11.

(Reproduced from water-colour wash-drawings made by Miss Gertrude Woodward.)

PLATE 8.

FIG. 1.—The large Selsey rostrate flint implement; dorsal surface. Drawn of the actual size of the specimen.

A. Scar of very large conchoidal flake removed from the left side of the rostral region.

1 and *2*. Scars of parallel ribbon-like flakes removed from the region to the right of *A*.

Cort. Portions of the original cortex of the nodule.

PLATE 9.

FIG. 2.—The large Selsey rostrate; ventral surface. Drawn of the actual size of the specimen.

Cort. Small area of cortex or original "bark" of the nodule.

R, *S*, *T*, *V*, *X*, point to "conchoidal ripplings" of the fractured surface, each letter pointing to the conchoidal fracture produced by a separate blow: some heavy, some light.

PLATE 10.

FIG. 3.—The large Selsey rostrate, right lateral surface. Drawn of the actual size of the specimen.

Cort. An area of cortex or original bark of the nodule (see fig. 1, right side, for the same area).

2, *3*, *4*. Scars whence ribbon-like flakes have been struck, the scar 2 is seen in the view of the dorsal surface given in fig. 1; scars 3 and 4 are noticeable for the conchoidal transverse rippling of the flint.

5 is the scar of a broader flake parallel to *2*, *3*, *4*, which is truncated by the well-marked conchoidal scar, *6*, of another shaping-flake.

PLATE 11.

FIG. 4 and 5.—The two hammer-stones found in association with the Selsey rostrate. Drawn of the actual size of the specimens. That shown in the upper figure (fig. 5) is less than half the bulk of the other. It is of pyramidal form, and is drawn so as to show the apex of the pyramid as the centre of the picture, with three large concave flake scars and a fourth convex area, radiating from it. The apex shows numerous small fractures due to its use as the striking surface. The larger hammer-stone drawn in the lower figure (fig. 6) has also been shaped by large flakes to a pyramidal form. In this drawing the apex forms the top of the figure, the stone resting on the opposite surface or base.

Reversal of Asymmetry in the Plutei of Echinus miliaris.

By HIROSHI OHSHIMA, Kyushiu Imperial University, Fukuoka, Japan.

(Communicated by Prof. E. W. MacBride, F.R.S. Received February 15, 1921.)

While engaged in artificial rearing of *Echinus miliaris* under the guidance of Prof. MacBride,* I have come across a number of abnormal plutei which had the hydrocœle developed on the right side instead of in its normal position on the left side. Such reversed larvæ were first found on May 31, 1920, when they were eleven days old (text-fig. 1). The "larval" body was quite normal both in size and shape, but the hydrocœle, stone-canal, axial sinus, madreporic vesicle, and amniotic invagination were all situated on the right side, so that the larva became a perfect mirror-image of the normal larva. Such larvæ developed further with exactly the same rate of growth as the normal ones, an echinus-rudiment being well developed on the right side, until at last, when a month old, some few of them passed metamorphosis. The young urchins (text-fig. 2) showed no features visible externally which differed from those of urchins metamorphosed from normal larvæ. A similar case has previously been described only by Runnström in two individuals found among artificially reared larvæ of *Strongylocentrotus lividus* ((9), pp. 2–3, 7–10; (12), pp. 419–24, Plate 14, figs. 12–16). In other classes of Echinoderms, auriculariæ with the hydrocœle on the right side were noticed by Müller many years ago ((7), pp. 101, 109; Plate 5, fig. 1), and a similar state of affairs in two plutei of *Ophionotus hexactis* has recently been discovered by

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* It is my pleasant duty here to express my extreme indebtedness to Prof. E. W. MacBride for his kind supervision on the present work, and to Sir Sidney F. Harmer for permission to use the British Museum (Natural History) Library. A more detailed account of this work will be published elsewhere before long.

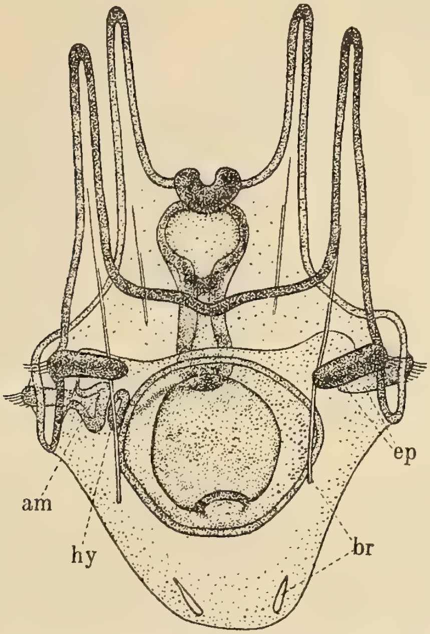

Fig. 1.—Ventral view of a reversed larva of *Echinus miliaris*, eleven days old ; drawn from life. ×100. *am*, amniotic invagination ; *br*, body-rod partly degenerated ; *ep*, ciliary epaulettes ; *hy*, hydrocœle.

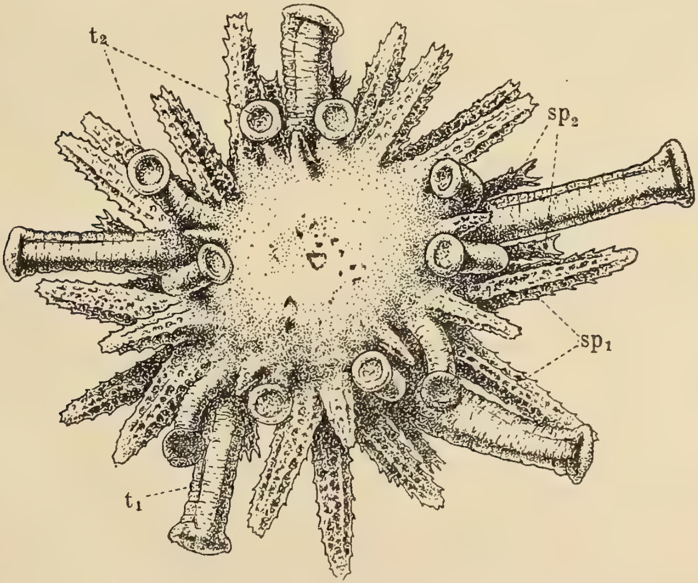

Fig. 2.—Young of *Echinus miliaris* metamorphosed from reversed larva, seen from actinal side ; thirty-two days old, and shortly after metamorphosis ; mouth not yet open ; drawn from life. × 100. sp_1, thick pointed spines ; sp_2, square-ending spines ; t_1, primary tentacles ; t_2, first paired tentacles.

Mortensen.* In both these cases the abnormal larvæ were found in nature, not reared under artificial conditions.

The purpose of our experiments was to repeat Prof. MacBride's method of artificial production of the double hydrocœle (6). Having been unable to get an uninterrupted supply of enough food, which consisted of *Nitzschia*, and from other causes unknown to us at present, we could not arrive at any satisfactory conclusion so far as the effect of the increased salinity is concerned. From both lots of cultures, treated with the "hypertonic" sea water as well as untreated, more than 10 per cent. of the larvæ exhibited the situs inversus, and much less number, 2 per cent. at most, developed the double hydrocœle.

There are at least three possible ways in which the situs inversus of the Echinoplutei may occur:—

1. It may be a case analogous to the reversed Gasteropods, as made known by Crampton and Conklin. Some unnatural conditions, unavoidable in the course of the artificial fertilisation of the sea-urchin eggs, may have changed the polarity of the latter, with the result that the structure of the larva assumes such a totally reversed situs (Conklin, (1), p. 585). It may be stated here in this connection that the phenomenon can hardly be regarded as due to heredity. Our cultures were obtained from three different parents. It seems highly improbable that we should find at least three congenitally reversed individuals (more than 4 per cent.) out of seventy sea-urchins which had been sent from Plymouth, and those three, those selected at random for their good eggs. The remainder were not tested. If the peculiarity were congenital, the occurrence of such reversed larvæ in nature would not be very rare.

2. Results of Runnström's experiments carried out with *Strongylocentrotus* and *Solaster* ((10), pp. 533–44, text-figs. 7*a*, 10; (14), pp. 471–73, text-figs. 16*a*, *b*) show that the larva developed from the right half of the egg or embryo of these Echinoderms had an indication of being a mirror-image of the normal form. If we compare this fact with Spemann's experiments on the twins and double monsters of *Triton*, in which the situs inversus occurred in most of the right side member ((15), pp. 390–93), we are led to suppose that the abnormal larvæ before us may have developed from those eggs or embryos which had accidentally undergone dissociation. Our abnormal larvæ were, however, neither smaller in size nor slower in development than the normal ones, so that the above supposition seems hardly

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* I have to acknowledge my hearty thanks to Dr. Th. Mortensen, who informed me of this discovery and kindly permitted me to mention it here.

acceptable in this case. On the other hand, we see in Gemmill's twin larvæ of *Luidia* ((2), Plate 2, fig. 13; Plate 3, figs. 19, 21) no indication whatever of a mirror-image on the part of the right side member. Thus, if it be true that the long-continued shaking caused the partial dissociation of blastomeres, as he interpreted the twin-formation, and in which interpretation there is room for some doubt, the result differs entirely from Runnström's.

3. The growth of a normally developing hydrocœle might have been arrested from some cause, and the right anterior cœlom, to compensate this defective development, produced a new hydrocœle on the right side. This seems to me most probable, if not exclusive, to have occurred in our case of abnormality, and it needs some further consideration.

From looking through the literature of the abnormal Echinoplutei and from my own observations, the following facts seem to me to have some important bearings on the formation of the abnormality in question :—

(*a*) Obliteration of the pore-canal (five cases in *Strongylocentrotus lividus* by Runnström, (9), p. 8; (12), p. 422; (9), p. 3; (12), p. 417, Plate 13, fig. 8*a*; (12), p. 413, Plate 13, fig. 4; (12), p. 415, Plate 13, fig. 5; (12), pp. 415–16. Two cases in *Echinus miliaris* by the writer, Culture No. 9, "treated"). This seems to happen in connection with the shifting of the pore towards the mid-dorsal line from its original position on the left side. The hydrocœle, thus deprived of its communication with the exterior, becomes arrested in its development, and then begins to degenerate quickly.

(*b*) Activation of the right anterior cœlom in its latent potentialities of producing a hydrocœle, to compensate the degenerating left hydrocœle. This new structure develops exactly after the manner of the normal hydrocœle on the left side. Actual observations of early stages of its formation are, however, unfortunately lacking.

(*c*) Restoration to the left hydrocœle of its external communication. This can take place either from the formation of a new pore-canal (two cases in *Strongylocentrotus lividus* by Runnström, (9), p. 8; (12), p. 422, Plate 14, fig. 15; (12), p. 416, Plate 13, figs. 7*a*, *b*), or from fusion of the left and right anterior cœloms into a single axial sinus (two cases in *Echinus miliaris* by MacBride, (6), p. 347, Plate 5, fig. 9; pp. 338, 348, Plate 8, fig. 18). The left hydrocœle will now continue to develop and differentiate further.

(*d*) Formation of an amniotic invagination on the right side. According to MacBride, this is due to the stimulating action of the right hydrocœle ((5), pp. 240–41; (6), p. 343), while Runnström is of the opinion that the invagination can be formed independently of the presense of a hydrocœle, though further development of both these structures are mutually dependent ((10), p. 305; (12), pp. 9–11, 13–14).

(*e*) Peculiar differentiation of the right posterior cœlom, to share with the hydrocœle and amniotic invagination in the formation of an echinus-rudiment, exactly as its left side fellow normally does.

(*f*) Formation of pedicellariæ on the left side. This occurs in the reversed larvæ (two cases in *Strongylocentrotus lividus* by Runnström (9), p. 2, (9), p. 10, (12), p. 422) and in some double-hydrocœle ones (*Echinus miliaris* MacBride (6), p. 343). They probably owe their existence to the echinus-rudiment developed on the opposite side. In the complete absence of hydrocœle from both sides pedicellariæ cannot usually be formed at all (exceptional cases are recorded by Runnström (11), pp. 269–70, text-figs. 33–35).

From these data above enumerated, if adequately combined, the following changes seem to be possible:—

Let us start with a young normal larva, in which hydrocœle, axial sinus, pore-canal, and dorsal pore are all formed on the left side. An amniotic invagination may already be formed on the left side, and the right anterior cœlom may be provided with a pore-canal.

Now, the dorsal pore of the left side happens to become obliterated, and is hence followed by the arrest of development of the hydrocœle and its subsequent degeneration. Two courses are here open:—

A. The right anterior cœlom begins to exhibit its unusual activity in producing a right hydrocœle, which acquires through a pore-canal a communication with the exterior.

B. The right anterior cœlom does not become active (presumably from want of sufficient nutrition). The result is the complete absence of hydrocœle from both sides.

The further fate of larvæ in which the course of events has been that indicated by A will be one of the following three:—

1. Appearance of a new dorsal pore on the left side, so that the left hydrocœle regains the power to develop further. If well fed, the hydrocœle on each side will continue to develop side by side, so as to give rise to a double-hydrocœle larva.

2. Fusion of the anterior cœloms of both sides takes place, so as to enable the left hydrocœle to communicate with the exterior and to continue further development. The result is also a double hydrocœle.

3. No reappearance of a second dorsal pore on the left side nor fusion of the anterior cœloms takes place. The left water-vascular system will consequently degenerate quickly, while the right one will develop like the normal left. *A larva with the situs inversus is the result.*

In both the courses of events indicated by 1 and 2 the following three conditions may possibly arise, according to the different stages at which the right hydrocœle had arrived, when the recovery of the left hydrocœle took place :—

(*a*) The recovery of the left hydrocœle takes place before the right hydrocœle attains a size equal to that of the left. The period during which the hydrocœle is deprived of communication with the exterior is very short. Under such a condition the larva in which the left hydrocœle is more advanced than the right will result. This is very frequently met with among double hydrocœle larvæ.

(*b*) The left hydrocœle recovers at the time when the right one has attained the size about equal to it. The larvæ developed under such a condition has two hydrocœles equal in their states of development. Such a case is less frequently met with than the former.

(*c*) The left hydrocele recovers late when the right one is already in a more advanced state than it. The period during which the hydrocœle is deprived of communication with the exterior is here very long. The result is that the larva has the left hydrocœle less developed than the right. Usually the hydrocœle cannot remain unchanged for so long a time after being deprived of its external communication. The case is therefore met with very rarely.

The above may not be the only ways of reaching the respective results, but probably are the commonest. Many modifications are naturally conceivable.

Whether the extraordinary development of a hydrocœle from the right anterior cœlom is to be regarded as a case of atavism or as other variation is, from the poor state of our knowledge at present, a matter of choice. MacBride ((5), pp. 240, 244 ; (6), pp. 341–45) and v. Ubisch ((16), pp. 444–45) are of the opinion that it is a case of atavism. Grave ((4), p. 43) made the objection to this view that it necessitates the admission of such an improbable fact that the Echinoderm ancestor had a double set of spines and dental apparatus. According to MacBride, the appearance of the right hydrocœle to a certain stage of development is an atavistic feature, but later the formation of the amniotic invagination and those calcareous structures is due to the action of a hormone emanated from the hydrocœle ((5), pp. 247–48 ; (6), pp. 342–43). If this peculiar ability has been acquired by the left hydrocœle during the later period of Echinoid history, as suggested by v. Ubisch, then how could it be transmitted to the right hydrocele now ?

Gemmill, discussing the causation of the double hydrocœle in starfish larvæ ((3), p. 72), introduces the idea of homœosis, which I prefer to the atavistic view. The difficulty of accepting the idea of homœosis in such a case, as maintained by Grave ((4), p. 45), can easily be overcome, because the development of the double hydrocœle is not a feature of adaptation to larval life, as he assumes it to be.

The unusual development of a hydrocœle on the right side when the left hydrocœle is arrested in its development resembles the well-known phenomenon in the regenerating claw of *Alpheus* (Przibram (8)). If the large claw of this crustacean is amputated, this will be replaced by a small claw, whilst the small claw on the other side, which has not been operated upon, will become a large claw. Przibram called this "compensatory hypertypy." Obviously these two cases are different in that (i), whereas in the Echinopluteus there is no trace of a right hydrocœle in normal condition, the small claw of *Alpheus* represents neither a rudimentary condition nor an early developmental stage, but is quite a functional organ; and that (ii), whereas the left hydrocœle of the Echinopluteus is arrested in its development during its very early stage, the large claw of *Alpheus* is removed after it has fully developed. With the sense widened to include these two cases, Przibram's term is desirable to be adopted here also.

The manner in which the situs inversus occurs in Echinoplutei resembles that found in the *Triton* larvæ, as produced artificially by Spemann ((15), p. 407). In both cases the abnormality starts with a "defective" development of a single organ—the gut in *Triton* and the hydrocœle in *Echinus*. There is, however, a difference between the two in the further courses in which other organs are affected. In *Triton* the other adjoining organs are simply displaced by that abnormal behaviour of the gut, while in *Echinus* a new hydrocœle appears on the right side, and a new set of calcareous structures, etc., are thereby induced to develop. The left hydrocœle can, if it regains its chance of further development, produce another echinus-rudiment, so as to give rise to a double-hydrocœle larva. Such a feature is very improbable to occur in the case of *Triton*.

My interpretation of the double-hydrocœle formation as described above cannot hold true in the case of starfish larvæ. Usually in the starfish larva the cœlomic vesicles on both sides fuse and communicate with each other at the anterior end, and the right dorsal pore, if present at first, gradually atrophies. Here in this case the obliteration of a dorsal pore has no influence upon the development and differentiation of the hydrocœle on the corresponding side. Under such different conditions I think that the occurrence among starfish larvæ of the situs inversus as is found in Echinoid larvæ will be extremely uncommon. Gemmill's interpretation as to the cause of the double-hydrocœle formation ((3), pp. 54-55) cannot, in its turn, hold true in the cases of the Echinoid and Ophiuroid larvæ, as was pointed out by

MacBride ((6), p. 326). One feature is, however, certainly common to the double-hydrocœle larvæ of these three classes of Echinoderms, namely, the temporary arrest of the growth of the left hydrocœle in an early stage, from some cause or other. And this occurs more frequently under artificial conditions than in nature.

What then is the external factor which causes the arrest of growth of the left hydrocœle in the Echinoid larvæ? It seems to me that the obliteration of the pore-canal is a direct cause of the arrest, and that obliteration of the canal is associated with the presence of too much diatom food and other micro-organisms in the culture jar, which arouses either mechanical or physiological disturbance in the normal development of the larva. The phenomenon in which the latent potentiality of producing a hydrocœle of the right anterior cœlom is provoked has been ingeniously compared by MacBride to artificial parthenogenesis ((6), p. 341). He thus regards the hypertonic sea water as one of the chief factors producing the double hydrocœle. This most interesting hypothesis is not as yet fully proved, and it is desirable that it should be set free from criticisms through further test.

Note by Prof. E. W. MacBride, F.R.S.

My friend Dr. Ohshima and I agree in our fundamental explanation of the development of two hydrocœles in the larva of *Echinus miliaris.* We both attribute this phenomenon to a latent power in the right anterior cœlom developing a hydrocœle, a power which is normally inhibited by the development of the functional hydrocœle from the left anterior cœlom. This latent power of the right side, which is also manifested in the larvæ of Asteroidea and Ophiuroidea, I believe to be an indication that the common bilateral ancestor of the Echinodermata had, corresponding to the hydrocœle, a paired organ equally developed on both sides of the body, and that, whilst the organ on the left side became further developed until it grew to be the water-vascular system and its appendages, the organ on the right side dwindled and disappeared.

Dr. Ohshima does not accept this explanation, but prefers to explain the development of a right hydrocœle as "homœosis." I think this is really to substitute a word for an explanation. What is meant by homœosis? The word was coined by Bateson (1894) to signify the phenomenon which appears in the meristic repetition of seriate organs, viz., that a segment sometimes acquires the characters of a member of the series preceding or succeeding it. But this phenomenon is now more fully understood; it depends on the formation of tertiary organs—as we may call them—under the stress of formative stimuli. Thus the proto-vertebræ of the developing newt Necturus are at first all alike, but that particular one will develop into a sacral vertebra which happens to come in contact with the pelvic girdle—and this may occur at slightly different levels on the two sides of the animal. Unless the right and left anterior cœlomic sacs of Echinoderm larvæ had been originally alike in their potentialities, it is difficult to see how the powers of one could have been transferred to the other, as they do not form parts of a series.

In the paper which I communicated to the Society in 1918, I gave reasons for believing that one means of procuring the growth of two hydrocœles was to subject the growing larvæ to the action of hypertonic water for a period of about a week. This result was obtained during the summer of 1917. During the summer of 1919 I repeated the experiments, and obtained results so entirely confirmatory of those obtained in 1917 that I did not think it worth while to publish them.

During the spring and summer of 1920, when Dr. Ohshima worked in my laboratory, attempts to procure similar results failed: we did, indeed, obtain a few larvæ with double hydrocœles, but these were found mostly in cultures which had not been treated with hypertonic water at all.

Dr. Ohshima is naturally sceptical as to my diagnosis of the cause of the appearance of the double hydrocœle. I have therefore to offer the following suggestions as to the reason for the divergence of the results of 1920 from those of 1917:—

(1) The experiments of 1917 were carried out in natural sea water, which up till that time was sold—as in pre-War days by the Great Eastern Railway Company—at a cheap rate in London.

After the spring of 1917 this service was stopped, and for further experiments we had to rely on artificially prepared sea water, which is not so good for the growth of the larvæ.

(2) Dr. Ohshima's work is based on the results of two cultures, one obtained during May and one during June.

The May culture was not a very vigorous one—in other words, the proportion of ripe viable eggs shaken out was small compared to the mass of unripe eggs, and the early development was not very vigorous, though ultimately quite a considerable number of larvæ completed their metamorphoses.

I suggest that in the case of this culture the reaction to hypertonic water, which I noted in the vigorous culture of 1917 did not take place.

(3) On the other hand, the June culture was an extremely vigorous one. When I examined the larvæ of that portion of it which had been exposed to the action of hypertonic water, a large proportion showed signs of the

beginning of the development of a right hydrocœle. Then, however, in spite of all our endeavours to keep it alive, the Nitzschia culture, which was used for food, died off, and the development of the Echinus larvæ was consequently arrested. In fact, all of those that had been exposed to the action of hypertonic water died, and the larvæ in the control culture were given up for lost. But two months afterwards Dr. Ohshima, returning after an absence, found many of these larvæ alive and in an advanced state of development, and amongst them he discovered, in addition to a certain number of right-handed larvæ, a number which had developed two hydrocœles.

The explanation of this phenomenon which I suggest, is that the long period of starvation had checked the normal development of the left hydrocœle, so that this had remained small, and when conditions improved the innate tendency of the right side to develop a hydrocœle was no longer inhibited by too great a development on the left side.

If this explanation is justified in Dr. Ohshima's specimens, the right hydrocœle would have been produced by the *negative method* of checking the development of the left side, whilst in my earlier experiments it was produced by the *positive method* of direct stimulation of the right side.

Dr. Ohshima quotes an observation of Runnström's to the effect that a larva had been observed by him devoid entirely of a hydrocœle, but with pedicellariæ developed on both sides. I suggest that Runnström has overlooked the presence of vestigial hydrocœles in this larva. As I proved in 1918 (6), when larvæ are starved so that the hydrocœle rudiment is completely absorbed, they develop pointed spines on both sides, but no pedicellariæ. I too, like Runnström, imagined that I had found larvæ with pedicellariæ on both sides, but no hydrocœle; but these, when mounted whole and closely examined, disclosed minute hydrocœles on both sides.

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The Energy Involved in the Electric Change in Muscle and Nerve.

By A. V. Hill, F.R.S.

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Considerable electromotive forces are produced by the activity of excited muscles or nerves—up to three or four hundredths of a volt—and it was conceivable that an appreciable amount of energy might be involved in the currents set up in the tissue by them. This paper contains an examination of the question.

In fig. 1 is shown a nerve fibre, on which rest electrodes (not shown) connected to an electrometer or galvanometer. Along the outside of the fibre is travelling, from right to left, a wave of negative potential, with velocity a cm. per second, having at any point distant x cm. along the nerve, and at time t seconds, a value y volts, as recorded on the electrometer, and shown (after the appropriate analysis) in the lower curve of the figure. We are not concerned here with the cause of this electromotive change, nor with what happens inside the fibre, but only with the physical results of it in an external circuit.

In consequence of the differences of potential existing along the length of the nerve, currents tend to flow, as shown by the full arrows and the strength of the current flowing at any moment between two neighbouring points, is proportional to the difference of potential between them. Thus the current in the external circuit *at* any point at any given moment is directly proportional to the slope at that point of the curve giving the E.M.F. of the

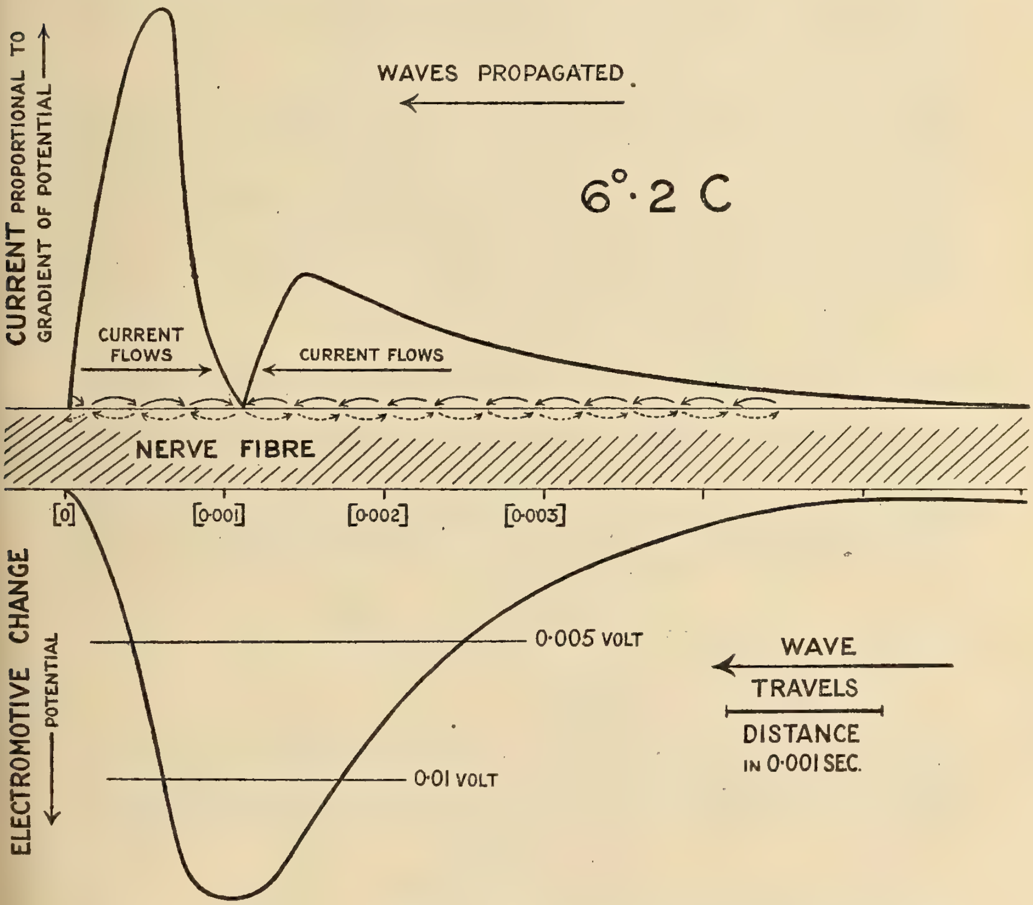

Fig. 1.—Sciatic nerve fibre, at 6·2° C., showing observed monophasic electric response (lower curve) moving from right to left with velocity about 10 metres per second, and consequent current distribution in nerve (upper curves). Black curved arrows show flow of current outside fibre: dotted curved arrows show conjectured flow inside fibre. The numbers in square brackets below the fibre represent times (in seconds) since the front of the wave passed the corresponding points.

electric change, and is represented graphically by the upper curve of the figure.

This current distribution should be regarded as sweeping along the nerve to the left with velocity a. It is probable that the currents observed on the outside of the fibre by experimental means return on the inside of the fibre, as shown by the dotted arrows of fig. 1, but, as the internal electrical resistance is unknown, and as the distribution of the return currents is a matter more or less of speculation, it seems advisable to consider only the effect of the currents in the external circuit, where the E.M.F. and the resistance can be accurately observed. The energy of the currents, as calculated below, is less, therefore, than the true amount by the unknown quantity involved in the return currents inside the fibre.

The most natural assumption as to these return currents is that they are similar to the external currents observed, the E.M.F. being located somehow in the walls of the fibre; in that case the total amount of energy involved in the electric change is double the quantity calculated below.

Suppose that the wave of negative potential shown in the lower curve of the figure is represented by the equation

$$y = f(t - x/a),$$

the function f being given by the observed form of the electric response. Let R be the resistance per unit length of a tissue, as measured by direct experimental means. The potential at a point x being $(t - x/a)$ at a moment t, the potential at a neighbouring point $(x + \delta x)$ at the same moment will be $f\{t - (x + \delta x)/a\}$, so that the current running in the small element of length δx between them will be

$$\begin{aligned} \mathrm{C} &= \frac{f\{t-(x+\delta x)/a\} - f(t - x/a)}{\mathrm{R}\delta x} \\ &= \frac{f'(t - x/a)}{\mathrm{R}a}, \end{aligned}$$

where $f'(z)$ is the differential coefficient of $f(z)$ with respect to z. Hence the heat produced in this element of tissue in time t, being $\mathrm{R}\delta x \mathrm{C}^2 \delta t$ joules, is equal to

$$\frac{f'^2}{4{\cdot}18\,\mathrm{R}a^2}\,\delta x\,\delta t \text{ calories.}$$

Integrating this with respect to x and t, in order to determine the total heat H produced in the whole length l of the tissue by the whole wave, we find

$$\mathrm{H} = \frac{1}{4{\cdot}18\,\mathrm{R}a^2} \int_{x=0}^{x=l} \int_{t=0}^{t=\infty} f'^2\,dx\,dt.$$

Now it is obvious that if the wave be propagated unchanged the same amount of heat is liberated at each point of the tissue: hence the value of $\int_{t=0}^{t=\infty} f'^2\,dt$ cannot depend upon x and must be equal to its value at any convenient point on the tissue, *e.g.*, at the electrode used in the experiments. Hence, from a curve relating electrical potential to time at one spot (as observed in a monophasic response), we can calculate this integral numerically: suppose that its value is A: then the total heat becomes

$$\frac{1}{4{\cdot}18\,\mathrm{R}a^2}\int_0^l \mathrm{A}dx = \frac{\mathrm{A}l}{4{\cdot}18\,\mathrm{R}a^2}\text{ calories,}$$

or, per unit length, $$\frac{\mathrm{A}}{4{\cdot}18\,\mathrm{R}a^2}\text{ calories.}$$

The value of R in this formula can be considerably decreased by immersing the muscle or nerve in a conducting fluid. I am informed, however, by Dr. E. D. Adrian that the potential difference of the action current as measured by an electrometer also depends very much on the amount of fluid adhering to the nerve, and that a large E.M.F. can be obtained only by using a fairly dry nerve; in fact, the external conducting fluid is acting as a short-circuit and lowering the E.M.F. between any two points on the tissue. It is probable therefore that no considerable increase in the production of heat will be caused by lowering the resistance between two points on the nerve by immersing it in a conducting fluid. We will consider only the case of a muscle or nerve in a reasonably dry condition.

Let us apply the formulæ deduced above to the case of the monophasic electric change of the frog's sartorius, as determined by Keith Lucas* for 8° C. and 18° C. The data are as follows:—

Secs.	8° C. volts.	18° C. volts.	Secs.	8° C. volts.	18° C. volts.
0·0050	0·0013	—	0·0094	0·0325	0·0144
0·0056	0·0032	0·0011	0·0100	0·0268	0·0107
0·0062	0·0089	0·0052	0·0106	0·0206	—
0·0069	0·0187	0·0156	0·0112	0·0140	0·0067
0·0075	0·0239	0·0213	0·0125	0·0070	0·0054
0·0081	0·0292	0·0251	0·0150	—	0·0048
0·0087	0·0322	0·0217			

These numbers have been plotted in curves and the values of A calculated. They are as follows:—

$$\mathrm{A}_8 = 0{\cdot}5, \qquad \mathrm{A}_{18} = 0{\cdot}35.$$

The velocity of the wave may be taken to be roughly as follows in the two cases:—

$$\mathrm{A}_8 = 140\text{ cm./sec.,} \qquad \mathrm{A}_{18} = 220\text{ cm./sec.}$$

These results are approximate only, as Keith Lucas does not give the exact distance apart of his electrodes in the case where he recorded the diphasic response. He states, however, that they were "as far apart as possible," and from his figure their distance cannot have been far from 2 cm. The time relations of the diphasic response recorded by him then give the above velocities.

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* 'Journal of Physiol.,' vol. 39, p. 220 (1909).

The resistance to an alternating current of a frog's sartorius muscle, after death, between electrodes placed directly upon it, is given by the following hitherto unpublished experiments, made by myself in 1913, at about 12° C., by the method described in a previous paper.*

(1)	No. of experiment	1	2	3	4	5
(2)	Distance between electrodes, centimetres	3·0	2·85	3·2	3·7	3·2
(3)	Mass of muscle between electrodes, grammes	0·183	0·147	0·205	0·241	0·207
(4)	Resistance between electrodes, ohms	13540	14860	12300	15910	12800
(5)	Resistance per centimetre, ohms	4510	5220	3850	4300	4000
(6)	Mass per centimetre, grammes	0·061	0·0515	0·064	0·065	0·065
(7)	(5) × (6)	275	270	247	280	259

The muscles being of different sizes, the conductivity should be proportional to the mass per centimetre, or, in other words, the resistance per centimetre multiplied by the mass per centimetre should be constant. That this is so is shown by the last row where this quantity is tabulated, We may assume therefore that a muscle 1 cm. long, weighing 1 grm., has a resistance of about 260 ohms at 12° C. Substituting this value with those preceding in the expression for the heat per unit length of muscle, viz., $H = A/4{\cdot}18\,Ra^2$, we find

$$H_8 = \frac{0{\cdot}5}{4{\cdot}18 \times 260 \times 140^2} = 23{\cdot}4 \times 10^{-9} \text{ calories per gramme,}$$

$$H_{18} = \frac{0{\cdot}35}{4{\cdot}18 \times 260 \times 220^2} = 6{\cdot}6 \times 10^{-9} \text{ calories per gramme.}$$

The quantity of heat liberated in a muscle twitch is of the order of 3×10^{-3} calories per gramme, *a quantity more than one hundred thousand times as great as the larger of the quantities calculated above.* The amount of energy associated with the electric change in a muscle is negligible therefore when compared with that liberated in the subsequent contraction.

The same formulæ may be applied to the case of a nerve. Dr. E. D. Adrian has kindly provided me with a record of a monophasic response of a frog's sciatic nerve at 6·2° C., which is the curve shown in the lower half of fig. 1. Here the calculated value of A is 0·38 (compare this with the value 0·5 given above for the sartorius muscle at 8° C.), and the value of *a* was about 10 metres, *i.e.*, 1000 cm. per second. Assuming that the specific electrical resistance of nerve is the same as that of muscle, an assumption which can cause no serious error, the value of the heat production at 6·2° C., per gramme of nerve, caused by the passage of a single electric change becomes,

$$H_6 = \frac{0{\cdot}38}{4{\cdot}18 \times 260 \times 1000^2} = 3{\cdot}5 \times 10^{-10} \text{ calories.}$$

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* A. V. Hill, 'Journal of Physiol.,' vol. 46, p. 457 (1913).

This quantity lies between 1 and 2 per cent. of the value calculated for the sartorius muscle at 8° C.

The precise values deduced above are, of course, of no importance as they can be changed considerably by small alterations in the experimental conditions. The interest lies in their *order of size*, not in their exact values. Consider the case of a nerve. In a previous paper* the production of heat (if any) in the passage of a single impulse along a nerve was shown to be not greater than about 5×10^{-8} calories per gramme. This is more than 100 times as large as the heat liberated by the electric currents produced by the nerve. Now *the only known accompaniment of the nervous impulse is the electric change*, and it is of importance therefore to realise that this electric change involves in itself only a very small liberation of energy, corresponding to a rise of temperature of less than one thousand millionth of a degree, or to the amount of work required to lift the tissue through fifteen millionths of a millimetre. It is natural, therefore, that no direct method should be capable of determining the heat liberated in the passage of a nervous impulse.

The electric change however, is, in a way, a relatively large effect, being quite easily shown on a galvanometer or electrometer, and capable of directly exciting other tissues ("rheoscopic frog"). Presumably, therefore, it is energetic enough to account for the sudden and temporary change of permeability required to initiate the muscle twitch. The facts: (*a*) that apart from the electric change there is no known accompaniment of the nervous impulse; (*b*) that down to 5×10^{-8} calories per gramme no production of heat occurs in the passage of an impulse; (*c*) that the energy involved in the electric change itself is almost inconceivably small; and (*d*) that the electric change is nevertheless sufficient to stimulate other tissues; tend to confirm the belief either that the electric change *is* the nervous impulse, propagated in some manner at present unknown, or that it is the immediate consequence of some physico-chemical change propagated as a wave with a very small degradation of energy. Unlike the mechanical response of muscle, the electric response of muscle and nerve is accompanied by practically no liberation of energy.

Summary.

An expression is given for the heating effect, in a muscle or nerve, of the currents produced by the electric response accompanying the propagated impulse. In a muscle the heat produced is not more than one hundred thousandth part of the energy liberated in a twitch; in a nerve it is of the order of size of $3{\cdot}5 \times 10^{-10}$ calorie or 0·015 erg. per gramme. It is concluded from the smallness of these quantities that no appreciable provision of energy

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* A. V. Hill, 'Journal of Physiol.,' vol. 43, p. 433 (1912).

is required in the propagation of the electric response, and that the physico-chemical change producing the response is the only factor involved in the propagated nervous impulse.

My thanks are due to Dr. E. D. Adrian and to Mr. R. H. Fowler, both of Trinity College, Cambridge, to the former for experimental results and critical suggestions, to the latter for help in the mathematical treatment of the problem.

A Method of Analysing Galvanometer Records.

By W. Hartree, M.A., and A. V. Hill, Sc.D., F.R.S.

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Croonian Lecture :—*Release of Function in the Nervous System.*

By Henry Head, M.D., F.R.S.

(Received April 22,—Lecture delivered May 5, 1921.)

It is a common experience that the manifestations of nervous disease may comprise both loss of function and some positive outburst of excessive activity. Thus, in many cases of hemiplegia, the paralysed limbs tend to be more or less rigid and the reflexes are greatly increased. This spasticity, although due to a destructive lesion, was attributed to "irritation"; the morbid agent, or the conditions it produced in the nervous system, were supposed to have an "irritative" effect upon the tissues, and this was expressed in the positive signs and symptoms.

But more than fifty years ago, Hughlings Jackson [11]* laid down the rule that destructive lesions never cause positive effects, but induce a negative condition, which permits positive symptoms to appear. This he applied to all morbid expressions of nervous activity; but his most striking instance was the motor condition in organic hemiplegia. He showed that it depended on two factors. First, there was loss of voluntary power, especially in the fingers, the parts most directly under cortical control; this was the negative effect of the lesion. Secondly, the positive symptoms consisted of that massive overaction spoken of as "spasticity," the result of the unrestrained energy of lower centres. It was not due, as was commonly supposed, to "irritation," but to release of such centres from the control normally exercised over them by those on a higher functional level.

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* Numbers in square brackets refer to List of References at end.

This doctrine, in its specific application to certain phenomena of hemiplegia, was widely accepted, especially in England; but it was not generally applied to the phenomena of nervous disease, and it is still the custom to attribute to "irritation" most of the excessive reactions produced by organic disease or injury.

All observers are agreed that the various centres of the nervous system form a hierarchy in which some exercise higher functions than the others; but the idea that an abnormal response may be an expression of the activity of these subordinate centres has not penetrated widely into neurological medicine. The phenomena of disease are thought to be purely adventitious; they are spoken of as "pathological," and are supposed to bear no relation, even remotely, to any mode of response which has previously existed in the individual or the race.

We believe, on the other hand, that in the gradual evolution of function, the reactions of the lower centres have been changed to suit fresh conditions. The more complex an organism, and the more efficiently it responds with discrimination to external forces, the greater will be the need for such readjustment. Older modes of response must be modified or suppressed; some are utilised in the newly acquired complex act, whilst others, under normal conditions, are prevented from playing a part. But wherever diminution or removal of a dominant group of functions leads to an excessive outburst of energy, the more primitive responses have not been abolished; for had they disappeared completely in the process of evolution, destruction of a higher centre would produce loss of function only, unaccompanied by signs of over-activity. Normally, they are suppressed because they would disturb the more discriminative responses of higher centres; but they still remain capable of revival under conditions demanding urgent and impulsive action.

Release of function, due to failure of higher centres to exercise normal control, can occur in two ways. Structural changes may so lower their capacity that they can no longer play a dominant part in some complex act, and suppress inappropriate manifestations from subordinate centres. In this case the morbid phenomena consist both of loss of function and over-action. This I shall speak of as "disintegration," for it involves the breaking up of an integrated series of responses.

Secondly, some reaction, normally dominated or suppressed, may break through owing either to the extreme vehemence of the stimulus or to diminution of the controlling energy, due to causes other than organic destruction. Here the whole of the morbid manifestations consist of positive signs and symptoms unaccompanied by loss of function. This I shall call "escape from control."

1. *Disintegration.*

Sherrington's physiological work on the reflexes [16], and especially his explanation of decerebrate rigidity, gave an experimental proof of Jackson's law of release of function. If the cerebral hemispheres are removed, or better still, if the animal is decapitated through the mid-brain, all four limbs fall into a condition of postural rigidity, due to prolonged spasm of certain voluntary muscles. The head is lifted and the tail may be stiffly curved upwards. This attitude is essentially adapted to resist the force of gravity and, if the limbs are passively flexed by the experimenter, considerable resistance is encountered. The knee-jerk assumes a characteristic form; on tapping the tendon a quick extensor movement is produced, but the limb does not return to its original position. Removal of controlling influences from above permits these postural activities of lower centres to manifest themselves unchecked, for the cortex is concerned mainly with the initiation or inhibition or movement and not with posture.

When the spinal cord is divided in man, or so gravely injured that conduction is destroyed, the lower extremities lie flaccid and atonic on the bed, in any position into which they may be placed. The urine is retained and the patient has no power of evacuating his bowels. All deep reflexes are abolished, and scratching the sole of the foot may either produce no movement of the toes or one that is feebly downwards; the receptive field for this reflex is restricted to the sole or even to the outer portion of the under surface of the foot.

Should the patient be young and strong, particularly if he remains free from cystitis, bed-sores, or fever, the deep reflexes reappear as the period of spinal shock passes away. The ankle jerk and then the knee jerk can be obtained; gradually the plantar reflex begins to assume a form characterised by an upward movement of the great toe. The field from which it can be evoked enlarges, and finally, in successful cases, the spinal cord becomes so excitable that stimulation anywhere below the level of the lesion may be followed by a characteristic upward movement of the toes. But this is a small portion only of the movements produced by nocuous stimulation; the ankle is dorsiflexed and the lower extremity bent both at knee and hip; not infrequently the abdominal wall contracts and every flexor muscle below the lesion may participate in an energetic withdrawal of the foot from harm.

Still more remarkable evidence of diffusion of afferent impulses within that part of the spinal cord which lies below the lesion is shown by the behaviour of the bladder and rectum. Not only can evacuation be facilitated by pricking the sole of the foot or by any manipulation which produces a flexor spasm, but passing fluid into the bladder or rectum may evoke an upward movement of the toe, accompanied by other flexor manifestations of the lower extremity ([9], p. 467, *et seq.* Riddoch [13]).

This condition has been spoken of as a "mass-reflex," because any afferent excitation below the level of the lesion is liable to produce motor effects not only in the parts normally thrown into action by such a stimulus, but also in organs that do not usually lie within the arc of its reflex influence. The bladder can be excited to action by stimulating the sole of the foot, and movements of the toes can be evoked by filling the bladder with fluid.

Removal of the influence of the higher centres from those of the spinal cord has led to a condition in which the outbust of energy, produced by appropriate stimulation, is not only excessive but unduly widespread. Judged, however, by the strength of the stimulus, there is nothing to show that the spinal centres have become more excitable; but they have been released from control and respond with a more massive and less discriminative reaction.

It has long been recognised that an apoplexy may be followed by painful sensations of extreme severity on the opposite half of the body. Largely owing to the researches of Dejerine and his co-workers (Dejerine and Egger [1], Dejerine and Roussy [2]), we now know that the lesion in such cases usually lies within the optic thalamus. Roussy ([14] and [15]), further elaborating these signs and symptoms, described a "syndrome thalamique"; five careful *post-mortem* examinations confirmed his diagnosis, and many others have since been reported which showed the truth of Dejerine's original generalisation (Long [12], Winkler and van Londen [20], Holmes and Head [10]).

The optic thalamus is one of the most intricate regions of the central nervous system and, since most pathological conditions in this situation do not produce selective destruction, the clinical picture presented by a thalamic lesion is usually extremely complex. But by a suitable choice of illustrative cases and by analysis of the symptoms they present, it is not difficult to discover the part played by this organ in sensation. (Head and Holmes [9], p. 551, *et seq.*)

First, it forms the receptive end-station for all sensory impulses. From this point they start on a fresh course to culminate in two centres, the cortex cerebri and the essential organ of the thalamus itself, each of which subserves a complementary aspect of sensation. Any sensory loss which may be produced by a lesion of the optic thalamus differs in no way from that caused by interference with afferent impulses, either as they enter this organ, or as they pass to the cortex by way of the internal capsule. But to these familiar defects may be added another factor, a tendency to react excessively to all potentially affective stimuli. The prick of a pin, painful pressure, excessive heat and cold, all produce more distress than on the normal side. At the same time, pleasurable warmth may evoke an unusually vivid response, and general affective states induce a more profound reaction over the disordered half of the body.

It was customary in the past to explain the pains and discomfort, which occur under such conditions, as due to "irritation." The lesion was supposed to "irritate" some part of the sensory path in the region of the optic thalamus and thus to produce pains and "hyperalgesia." But this conventional hypothesis is insufficient to explain the facts observed during the clinical course of those cases.

In the large majority of instances, the lesion proved to be of vascular origin and consisted of a hæmorrhage or softening. Now, such disorders of the central nervous system usually arise more or less suddenly; provided the destruction is not progressive, they tend to produce the maximum loss of function at the time of, or shortly after, their onset. Subsequent progress on the part of the patient is always accompanied by a certain amount of recovery. But in this group of thalamic cases the pains and over-reaction come on as a rule during the period of restoration of function, frequently a considerable time after the "stroke" has occurred, and they usually last unaltered for years. Moreover, the response to pleasurable stimuli may also become excessive, a condition entirely incompatible with the existence of an irritative process capable of evoking pain and discomfort.

Here, again, a gross organic lesion is followed, not only by loss of function, but also by positive over-action. This is due to the release of the optic thalamus and neighbouring parts from the control normally exercised by the activity of the cortex cerebri. On the afferent side, the essential organ of the thalamus is the centre for pain, heat, cold, and other affective aspects of sensation. When, therefore, it is freed from the restraining influence of the cortex, every stimulus capable of acting on this centre produces an excessive effect on the abnormal half of the body. Disintegration with release of function and not "irritation" is the cause of thalamic over-reaction.

From the earliest days of our work on the afferent nervous system we recognised that, when the skin was deprived of certain aspects of sensibility, the response to those which remained might become peculiarly vivid. Reaction to a prick was abnormal and excessive; the patient complained that it was more painful, although measurements showed that sensibility to this form of stimulation was not increased.

When such a nerve as the median or ulnar is divided, the extent of the insensibility to pricking is usually less than that of the anæsthesia to light touch (see figs. 2 and 3). Some fourteen days or more after the injury, this intermediate zone begins to behave in a peculiar manner. If the point of a pin is dragged across the skin from normal to affected parts, it is said to be "more painful" or to "hurt more" as it passes over the portion of the palm sensitive to pricking, but not to the lighter forms of tactile stimulation. This would usually be spoken of as "hyperalgesia"; but measurements have shown that the threshold is raised to cutaneous painful stimuli, and that, although the response is excessive, this area is in reality one of diminished sensibility.

The phenomena exhibited by a part of the skin, sensitive to pain, heat and cold, but deprived of some of the higher elements of sensibility, could be worked out in greater detail during the course of the experiment on my arm. Within a little over six weeks, after division and reunion of my radial and external cutaneous nerves, sensibility began to return; seven months after the operation, the back of the hand was sensitive to pain, to cold, to heat and to any form of contact which moved the hairs. But the sensation experienced was peculiar and quite unlike any reaction from normal parts; for, although less easily evoked, it was unduly vivid. The pain of a prick was intolerable; cold was said to be colder, and a suitable warm stimulus produced a more actively pleasant effect than over the normal skin; during this stage of recovery a high threshold was associated with a brisker response ([9], p. 258, *et seq.*).

The sensation aroused within the affected area had certain other peculiar characteristics. It radiated widely and was not confined to the neighbourhood of the stimulated spot. Portions of the affected area seemed to be linked up together, so that stimulation of the one evoked a sensation of the same specific quality referred to the other. Thus, brushing the hairs, pricking, or the application of heat and cold to the neighbourhood of the index knuckle, caused a diffuse outburst of the appropriate sensation over the dorsal aspect of the thumb. Moreover, the response was massive, diffuse, and bore little relation to the measured strength of the stimulus, provided it was effective ([9], p. 297).

Trotter and Davies ([18], p. 121), who divided seven nerve trunks in different parts of their bodies, write as follows of these peculiar manifestations:—"In addition to their directly practical importance, intensification (over-reaction) and peripheral reference are matters of great theoretical interest. To the subject of them they present a series of sensations entirely new and entirely outside his previous sensory experience, and yet they are surprisingly definite and unmistakable. No one who has not experienced them can appreciate the intense vividness with which they present themselves to the subject; and the investigator with no direct knowledge of them is likely even to be wearied by the importance their brilliance makes them assume in the subject's mind."

It has long been known that certain minute spots in the normal skin are especially sensitive to heat, to cold and to pain, although there was reason to believe that they were not the cause of all its sensory functions (Blix, Donaldson, von Frey). We were able to show that this punctate mechanism was responsible for the peculiar mode of reaction present during the first stage of recovery. For, whenever a part of the affected area became sensitive to heat, to cold, or to pain, one or more of these specific spots were discovered within it. At this stage, sensibility to contact was due solely to end-organs at the base of the hairs, and, if my hand was carefully shaved, I no longer responded to cotton-wool moved lightly over the surface.

All the organs of this punctate mechanism possess certain characteristics in common. They regain their function with great rapidity after the peripheral nerve has been successfully united and, for many months, the form assumed by the reaction of the skin to contact, to pain, to heat and to cold, shows that they alone are responsible for any sensibility it may possess. The response to a stimulus capable of exciting one of these organs is not strictly graduated by its intensity, but is arranged more nearly on an "all or nothing" principle.* So long as the stimulus is effective, it matters little how cold or how hot it may be; organs of this class indicate its quality, not its intensity.

A natural consequence of this mode of reaction is the overwhelming importance of the extent of surface covered by the stimulus rather than its intensity. For it is obvious that, if one cold-spot can be excited to vigorous activity by 20° C., a stimulus at the same temperature, sufficiently extensive to cover many cold spots, will seem to be colder, although in reality its intensity is the same. Thus, whenever sensation depends solely on the response of this class of end-organs, the extent of the stimulus is of greater importance than its intensity.

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* In the protopathic response there is undoubtedly a "more or less-ness," which is of the same nature as that denoted by the term intensity; it is not, therefore, strictly an "all or nothing" reaction. But variations in the intensity of the stimulus are of less importance to the sensation evoked than the extent of surface or number of end-organs it affects. So much is this the case that in the experiments on my hand, a cold object covering several cold spots, but of higher temperature, caused a colder sensation than an ice-cold rod applied to one spot only. Weber's law, and other expressions of exact quantitative relations between stimulus and sensation, do not hold for states of protopathic sensibility ([9] pp 306–311). It is the business, therefore, of the central nervous system to replace these impulsive responses by more discriminative sensations.

A peculiarity of this reaction, from any part of the skin innervated by this punctate system only, is the diffuseness and wide radiation of the sensation evoked. When a hair-clad part, such as the back of the hand, is stroked with cotton-wool, an intense "tingling" and "itching" is produced over widely remote parts of the affected area. This disappears when the skin is shaved, and is due to movement of the shafts of the hairs; it is not a function of the skin as a whole. In the same way, each effective hot, cold or painful stimulus produces its specific sensation, which is referred to the same remote parts of the affected area, irrespective of any difference in quality ([9], p. 302).

Thus an area, obviously defective in sensibility, is found to respond with extraordinary vividness and with a widespread massive sensory reaction. These manifestations grow steadily stronger throughout the first stage of recovery, with the gradual return of sensibility to pain and the increasing number of heat- and cold-spots.

Throughout this period the more discriminative aspects of sensation have been absent or very defective. But as soon as they begin to return, the mode of reaction changes. The diffused sensations give place to a more strictly localised response; radiation no longer occurs into remote parts of the affected area. At the same time, the power returns of distinguishing two compass-points, applied simultaneously. This is essentially an ability to recognise objects in two dimensional space, and it is this discriminative power which checks and controls the diffuse character of the more primitive method of response. The impulsive reaction of the heat- and cold-spots gives place to one graduated more closely according to the intensity of the stimulus, as the affected part becomes sensitive to temperatures which lie in the middle of the scale (27° to 38° C.).

Another remarkable change, due to the restoration of the more discriminative aspects of sensibility, is shown in the return of adaptation to different temperatures. Under normal conditions, if the hand has been exposed for some time to warmth, a stimulus at 29° C. may seem to be "cool," whilst, conversely, if it has been adapted to cold, the same object may appear to be "warm." So long as the skin is actively innervated solely by the punctate sensory mechanism, no such adaptation is possible within normal limits, and any effective thermal stimulus appears to be hot or cold, irrespective of the temperature to which the part has been previously exposed ([9], p. 292).

How completely these changes in the form of the response are due to recovery of control by a dominant system over the activity of one or more primitive functions was shown by the behaviour of my hand when exposed to the injurious effects of cold. The higher forms of sensibility are liable to be unfavourably affected by the general action of external cold, especially before they have been completely restored. At a time when almost the whole of the back of my hand had so far recovered that referred sensation could no longer be produced, the palm was placed upon ice; radiation and reference reappeared as vividly as of old, and the hand was thrown back for a time into its previous condition. The newly recovered activity of the high-grade system was disturbed by the cold, and impulses from the more primitive mechanism, previously inhibited, now passed on to reach consciousness uncontrolled.

This control may be exerted even by impulses from the healthy skin in the neighbourhood, if adjacent normal and abnormal parts are stimulated simultaneously. When a cold tube was placed so that it fell just within that part of the dorsum of the hand which was in a purely protopathic condition, a vivid referred sensation was always experienced in the thumb. But when the base of the tube fell partly within the affected area and partly over the adjacent normal skin, reference to the thumb was abolished. The only sensation produced was one of coldness around that part of the back of the hand in contact with the tube.

If this massive and ill-defined response were due to structural changes inherent in the act of recovery, it is difficult to see why it is not always present, whenever nerve fibres conducting painful and thermal impulses undergo regeneration. Occasionally it happens, by a fortunate chance, that after division of cutaneous nerves some part of the denervated area retains its discriminative sensibility to the lighter forms of tactile stimuli, though insensitive to prick, heat, and cold. With the restoration of these aspects of sensation, pain-, heat-, and cold-spots make their appearance; but under such conditions, the response is not unduly vivid, diffuse, or referred to remote parts. Recovery within an area already endowed with the more discriminative forms of sensibility is not accompanied by the phenomena we have attributed to loss of control ([9], p. 287). If over-reaction were due to irritation of "pain-fibres" in the course of regeneration, it should be independent of the coincident presence or absence of other sensory activities; but this is not the case.

Moreover, it is important to remember that this over-reaction is limited to areas which have lost the more discriminative aspects of tactile sensibility. The cutaneous fibres of the ulnar nerve, which conduct painful impressions, extend into the "median area" on the radial half of the palm. In a similar manner, a varying portion of the so-called "ulnar area" is innervated by fibres from the median. The supply of these two nerves overlaps considerably as far as painful sensibility is concerned. But the extent of the over-reaction, which may follow division of one or other of these peripheral nerves, is strictly limited to the borders of loss to the lighter degrees of tactile stimulation; it does not spread beyond them on to those parts of the

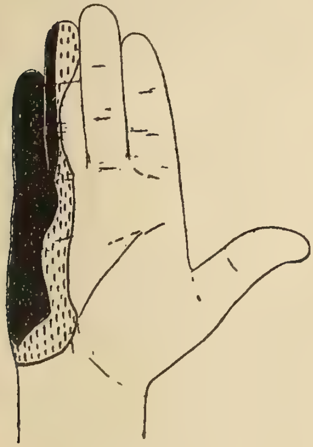

FIG. 1.

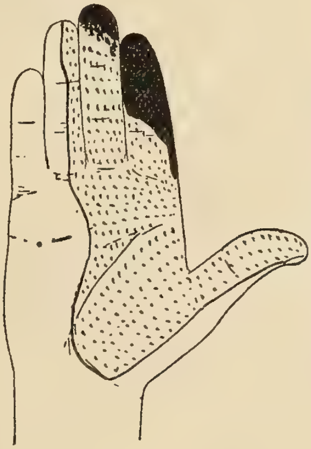

FIG. 2.

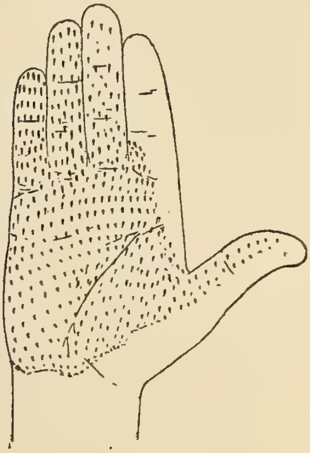

FIG. 3.

FIG. 1.—To show the effect produced by surgical division of the ulnar nerve. (From the same patient as fig. 3; the tenderness has disappeared.) The black area corresponds to the complete cutaneous analgesia. The dotted area represents the parts in a condition of protopathic sensibility; the surface was insensitive to the lighter degrees of contact, such as cotton-wool, and two points of the compasses could not be discriminated within normal limits. If a pin was dragged lightly across the palm from normal to abnormal parts it was said to "hurt more" when it passed to the ulnar side of this border.

FIG. 2.—To show the extent of the changes in cutaneous sensibility which may follow division of the median nerve. The black area corresponds to the complete cutaneous analgesia. An example has been chosen where the extent of this total loss was comparatively small. The dotted area represents the parts in a condition of protopathic sensibility; this does not extend beyond the limits of the loss to the lighter degrees of tactile stimulation.

FIG. 3.—To show the extent of the superficial tenderness on the palmar aspect of the hand, due to irritation of the ulnar nerve caused by a bullet wound of the forearm (Head and Sherren [9], p. 210, *et seq.*). The limits of this tenderness could not be defined exactly as it merged into parts that responded normally to the point of a pin dragged lightly across the surface. All tenderness disappeared after division of ulnar nerve (*cf.* fig. 1).

palm which we know to be supplied with "pain-fibres" from the injured trunk.

When, however, excessive reaction to prick is really due to irritation of a peripheral nerve, as in some cases of causalgia (fig. 3), the extent of the cutaneous tenderness is not limited by the loss of tactile sensibility, which is often slight; it spreads across the palm on to all those portions innervated from the injured nerve, and can be abolished by transecting it completely ([9], pp. 160 and 212).

I have attributed these morbid conditions, where gross loss of function is accompanied by unduly vivid reactions, to removal of some influence from above on the activities of lower levels of the nervous system; this doctrine has been widely opposed. Many hold that, when a complex function is broken up, the resultant positive manifestations are purely adventitious, and throw no light on the inherent reactions of the lower centres. Morbid changes lead to disordered action, of which "irritation" and excessive responses form an integral part.

But there can be no doubt that the postural rigidity of the decerebrate animal and the spasticity in hemiplegia express the activity of centres, which have remained unaffected by cerebral destruction. In the same way, the excessive responses to affective stimuli, which appear when the optic thalamus has been released from cortical control, and the mass-reflex obtained from the lower end of the spinal cord demonstrate the mode of reaction of these centres, when they are no longer dominated from above.

I have included in the same category the condition we have called "protopathic" sensibility,* which may appear after complete division of a peripheral nerve in parts of the affected area which remain sensitive to pricking, but are anæsthetic to light contacts; it is also specially evident during the first stage of functional recovery under identical conditions of sensory dissociation. For in such circumstances impulses arising in the punctate afferent mechanism can exercise their influence on the central nervous system, unchecked by the more discriminative impressions; these are either absent or have not yet been fully restored. Whatever explanation is given for such abnormal forms of sensation, there can be no doubt that in their vividness and indiscriminative nature they represent some want of higher control.

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* The terms "protopathic" and "epicritic" are applicable solely to forms of cutaneous sensibility, revealed by destructive lesions of the peripheral nervous system, and should not be employed for the products of dissociation at higher levels. All afferent impulses undergo regrouping, according to their potential sensory qualities, at the first synaptic junction. For instance, those due to painful impressions from the surface of the body are combined with equivalent impulses, produced by excessive pressure on deep end-organs, and travel up the same specific tract as far as the optic thalamus. In the same way the products of thermal stimulation, arising in the "protopathic" or "epicritic" mechanism of the skin, are sorted and re-grouped according to their power of arousing sensations of heat or cold. The optic thalamus forms the centre for qualitative and affective modes of sensation, whilst the so-called "sensory" cortex is occupied with its spacial and discriminative aspects. When the thalamus is freed from control, the abnormal half of the body reacts excessively to stimuli capable of evoking a response, whether pleasant or unpleasant, and in this way resembles a part in a condition of protopathic sensibility. But thalamic over-action is produced by the disintegration of a highly complex group of sensory functions, whilst protopathic sensibility is due to the uncontrolled action on normal centres of a set of impulses, heavily charged with feeling-tone and capable of inducing impulsive reactions.

All these examples of over-action, associated with gross loss of function, show certain primitive characteristics. They are not fortuitous perversions of a normal function, as some have contended, but reveal to a certain degree the mode of action of the lower centres before they were dominated from above. The reaction is massive and exceeds any normal response, both in vividness and extent; it is stereotyped, predetermined, and the same manifestations can be evoked from an abnormally wide receptive field. Moreover, it depends less upon the intensity of the excitation than on the amount of surface occupied by the stimulus, provided it is effective.

Thus, when a portion of the spinal cord is completely cut off from higher control, scratching the sole of the foot, or indeed any part below the level of the lesion, may produce spasmodic movements of the whole lower extremity and even of the abdomen. But this is not the only effect; bladder and rectum can be excited to evacuate their contents prematurely, and a profuse outburst of sweating be evoked by any nocuous stimulation. With the return of signs indicating that the higher centres again exert their influence on the lower end of the spinal cord, this want of differentiation ceases; scratching the sole of the foot no longer produces an effect upon the bladder and rectum, and the form assumed by the muscular response again begins to depend upon the situation and nature of the stimulus.

In the same way, on the afferent side, any effective stimulation of a protopathic area causes a vivid diffuse sensation, not uncommonly referred to remote parts at a distance from the place to which it is applied. The "more or less-ness" of the response is determined to a greater degree by the extent of the stimulus than by its intensity. The end-organs of the punctate system in the skin act as a warning mechanism; any form of excitation capable of producing a sensation leads to a highly impulsive but feebly discriminative reaction.

The abnormal vividness of a protopathic response applies not only to painful but also to all potentially unpleasant stimuli; the itching, produced by brushing the hairs of my hand, was more intolerable than any normal sensation. Pleasurable stimuli also evoke an excessive response, and the pleasure which accompanies moderate excitation of the heat-spots far exceeds that obtained from general stimulation of the normal skin with the same temperature. This exaggeration of the effect produced by potentially pleasurable stimuli forms a prominent feature in some cases of thalamic over-action, and has not been dealt with adequately by any of our critics. It cannot be explained by "irritation," nor is it a fortuitous perversion of some normal function. On the other hand, it falls into line with all the other phenomena we have attributed to loss of control.

Integration is the main business of the central nervous system, and within it the diverse effects produced on the living organism by external forces are sorted, combined and inhibited. Should the functions, which have been disturbed by an organic lesion, belong to the same level as those which are unaffected, the loss of one group has no material effect on the other; there is none of that over-action so common, when dominant centres are destroyed. When the lesion is situated in the lateral columns of the spinal cord, sensibility to heat and to cold may be disturbed independently; but isolated anæsthesia to heat over a certain part of the body is not accompanied by over-reaction to cold or *vice versâ*. At this point in their course through the nervous system, the impulses underlying sensations of heat and cold have already undergone qualitative integration; they run side by side in separate tracts and can be disturbed independently. Here they are equipollent; no one sensory activity is dominated by the other, and structural changes produce loss of sensation unaccompanied by excessive responses.

If, however, the factor eliminated by the lesion has exercised some control over the functions which still remain intact, phenomena may appear which were inhibited under normal conditions. Suppose half the scholars are absent from a class, the sum total of its vital energy is diminished, but discipline is maintained unimpaired; absence of the master, on the other hand, leads inevitably to disorder.

But it would be wrong to suppose that removal of a dominant mechanism reveals the reactions of a phylogenetically older organ in all their primitive simplicity. The integrative activity of the higher centres has profoundly modified the functions of those below them in the neural hierarchy; some have been caught up to take part in the new complex, whilst others are held in check or inhibited. Thus, the reactions obtained under favourable conditions from the divided spinal cord in man are more specifically flexor than in the dog or cat. For in the lower animals the specialisation of function is less complete and, when disintegration occurs, the separated spinal cord still retains certain functions which in man have mostly been assumed by higher centres. No flexor response is so free from postural characters as that which can be obtained from the lower portion of the human spinal cord after complete division. In the same way, the reactions of the optic thalamus in man, freed from cortical control, are an almost perfect expression of the non-discriminative aspects of sensation.

2. *Escape from Control.*

So far the phenomena I have described were due to disintegration of a complex function, normally carried out by the co-ordinated activity of centres at different levels of the nervous hierarchy. Before two such centres can work harmoniously, their various activities must be integrated, and during this process some impulses are inhibited, whilst others are facilitated. Of two antagonistic reflexes one becomes dominant, whilst the other is suppressed, according to circumstances, which are often more dependent on past events than on the immediate character of the stimulus. The issue of a conflict between opposing impulses is not inevitable; it is always possible for an unexpected or "abnormal" reaction to make its appearance, owing to some change in functional balance (Sherrington [16], p. 135).

The following experiment shows how completely one group of afferent impulses may inhibit another under perfectly normal conditions. On carefully exploring the back of the hand two or more cold-spots can usually be discovered in close proximity to one another. Stimulate any one of them by means of a metal rod, with a blunt end of not more than 1 mm. in diameter, heated to about 45° C.; the sensation will be one of coldness, as if the rod had been dipped in cold water. This is the familiar phenomenon of "paradox cold." Take a flat-bottomed metal tube containing water at about 45° C., of such a size that it will cover several cold-spots together with the skin around them. Lay this on the hand and you will experience a pure sensation of heat; and yet observation teaches us that this temperature is capable of evoking a sensation of cold, if it is applied to each of the cold-spots individually. Under normal conditions, however, the effect produced by stimulating the cold-spots is dominated, before it can affect consciousness, by the coincident impulses due to action of the same external physical agent on the end-organs which respond to heat.

The older investigators believed that an impulse, once started in specific end-organs, travelled unchanged to the highest receptive centres, to evoke a response corresponding in quality and degree to the sensory attributes of the physical stimulus. Such a theory ignores the fact that 45° C. is an effective stimulus to the heat-, the cold-, and the pain-spots, and yet, under normal conditions, produces a sensation of pleasant warmth only. Between the impact of a physical stimulus on the peripheral end-organs and the simplest changes it evokes in consciousness lie the various phases of physiological integration. We are accustomed to think of the external stimuli we employ in scientific investigation as if they caused unitary effects; but in reality they produce a multitude of diverse physiological reactions. The simplest physical condition acting on the peripheral nervous system may produce afferent impulses which are incompatible from the point of view of sensation. But the stimuli of daily life are not simple; they are extremely complex and give rise to a multitude of diverse impressions. These must be sorted and regrouped; some are facilitated, others are suppressed before the final sum is presented to consciousness. Many afferent impulses remain on the physiological level and never form the basis of consciousness; they are destined to control reflex activity or to co-ordinate movements of the body and limbs.

We must not forget that the larger number of afferent impulses, arising in peripheral end-organs, do not travel the whole length of the central nervous system. Their essential task is to excite and co-ordinate reflex action without of necessity giving rise to sensation, and the earlier phases of integration are undoubtedly adapted to this end. Such co-ordination could not occur if there was no qualitative selection of afferent impulses at the receptive junctions of the various reflex arcs. The main function of the intramedullary receptors is to increase the excitability of the reflex arc for one kind of stimulation and to diminish it for all others. This ensures a selective response to the complex results of peripheral excitation, and at the same time precludes many afferent impulses from influencing consciousness. Thus, for example, visceral insensibility is a function of the specific reaction of receptors, and not the consequence of an absence of afferent impulses.

Each specific group of receptive organs within the central nervous system works on the same principles; it accepts those elements of a massive reaction which can excite it to activity and rejects those to which it cannot respond. At the same time, there is evidence to show that the synaptic junctions are subject to those refractory states and phases of heightened activity characteristic of all neural action.

These three processes, selection of certain impulses, rejection of others, together with biphasic states of the receptive mechanism, are sufficient to produce coherent reactions on the reflex levels. Here all functions are simple, but at the same time highly organised. The response is rigidly predetermined within certain limits; there is little choice, and the answer is physiologically inevitable. In strict accordance with such an arrangement, we find that the first sorting of afferent impulses within the spinal cord is concerned mainly with their redistribution according to quality. Those capable of exciting pain are gathered together, whatever their peripheral origin, and all those which can evoke sensations of heat or of cold are combined in two specific groups.

After the multitudinous impulses produced by the impact of external and internal forces on the organism have carried out their reflex and co-ordinating functions, the remainder may pass on to excite sensations. These form an essential element in conscious acts, and here the rigidity of response, so characteristic of reflex activity, would be disastrous. The limited freedom of the lower levels must be expanded, so that the physiological reaction may become less inevitable and more elastic.

Moreover, the sensations to which these impulses can give rise are frequently incompatible; a temperature of about 45° C. stimulates at one and the same time organs capable of evoking pleasant heat, cold, and pain. These sensory and affective states cannot co-exist in consciousness; the struggle between the diverse groups of afferent impulses takes place, therefore, on the physiological level, and the victor alone appears as a sensation.

The simplest sensory act is the result, not only of prolonged qualitative integration, but of a constant struggle for physiological dominance. Under normal conditions the result may be inevitable; but there are many cases especially within the visceral field, where no general rule can be laid down, and the response depends on the patient's idiosyncracy or general resistance. Moreover, his usual reaction may become changed in consequence of some functional alteration in the activity of the central nervous system; impulses may then excite sensation, which normally would have remained outside consciousness.

The following observation, made on my hand in the later stages of regeneration, reveals the struggle for dominance amongst the various afferent impulses excited by thermal stimulation. The dorsal portion of my thumb had so far recovered that contact with a large vessel containing water at 40° C., produced a pleasant well localised sensation of warmth. But, at the same time, the skin in the neighbourhood of the index knuckle was in a pure condition of primitive sensibility, and a cold tube applied to this area evoked a diffuse and vivid outburst of cold referred to the back of the thumb. It was, therefore, possible to stimulate the skin of the thumb with heat, and simultaneously to evoke a sensation of cold referred to the same area. As soon as this was brought about all sensations of heat and cold ceased in the thumb and were replaced by discomfort and pain. When, however, the cold tube was removed from the neighbourhood of the index knuckle, the direct effect of the hot stimulus in contact with the thumb reasserted itself as pleasant warmth and the pain disappeared.

The behaviour of the normal glans penis ([9], p. 274) forms another excellent illustration of such inhibition. It is not uncommon to find that the punctate end-organs with which it is endowed are not uniformly distributed; the tip of the glans around the meatus may be devoid of heat-spots, but sensitive to cold and to pain. In such a case we carried out the following remarkable experiment. The end of the penis was dipped into a glass containing water at 40° C.; since no heat-spots were present and this temperature has no effect upon the cold-spots, the only sensation evoked was a peculiarly disagreeable pain. When, however, the temperature of the water was raised to 45° C., pain was to a great extent displaced by a vivid sensation of cold, due to stimulation of the cold-spots. Instead of increasing the discomfort an elevation of temperature ceased to be strictly painful because of the appearance of the specific sensation of "paradox cold." But around the corona the penis is always well furnished with heat-spots in addition to those for cold and for pain; as soon then as the water at 45° C. covered the corona without reaching the foreskin, both cold and pain disappeared, giving place to an exquisitely pleasant sensation of heat.

In none of these cases was the process of selective inhibition in any way conscious. The sensation evoked was a definite one of heat, of cold, or of pain; but it was the final issue of a struggle between incompatible and mutually antagonistic afferent impulses. The whole process takes place on the physiological level, and would have remained incomprehensible, had it not been for our knowledge of the phenomena of sensory dissociation.

Many impulses capable of forming the basis of a sensation are prevented under normal circumstances from reaching the highest centres; or, if their forward path is not completely barred, they pass on in a profoundly modified form, in consequence of the concurrent activity of other sensory end-organs. The utility of this arrangement is obvious, especially with those impulses which underlie discomfort or pain.

Pain is the oldest defensive reaction and potentially unpleasant stimuli are the basis of most primitive reflexes. It is, therefore, most important that the impulses they evoke should be diminished in extent, or actually inhibited in favour of those sensory impressions more capable of leading to discriminative action. The mechanism underlying the production of pain and discomfort must, however, remain in full physiological activity, so that it can play its part in defence of the organism, when noxious stimuli reach a high grade of intensity.

Thus, afferent impulses from the viscera do not normally enter consciousness. The eupeptic knows nothing of the processes of digestion beyond a certain gentle sense of well-being. But under abnormal conditions afferent impressions from the internal organs are capable of arousing pain and discomfort. These parts are probably innervated, like the glans penis, from the deep and protopathic systems; but unlike the glans their sensibility is extremely low. All power of conscious response to heat and cold is certainly absent from the greater part of the stomach and intestines, and pain cannot be produced by burning, cutting, and the prick of a pin or other sharp instrument. Internal surfaces are unable to respond to artificial stimuli to which they have never been exposed during the life of the individual or the race. But the hollow viscera, such as the stomach and bladder, react briskly to changes in tension which are their natural mode of stimulation.

Even if a stimulus is capable of exciting these sheltered parts of defective sensibility, it does not usually produce a sensation on account of the resistance of the nervous system to such potentially painful impressions. But a sensory response may be evoked whenever these visceral impulses become sufficiently strong to overcome the inhibition, or the forces opposing their passage are in any way diminished. Once the path has been opened and the dominance of the higher reflexes overcome, a weaker visceral stimulus will be followed by sensation; to this diminished resistance is due the production of pain from otherwise inadequate causes, where visceral irritation has been long continued.

Thus, under favourable conditions, afferent impulses from the viscera are capable of arousing pain and discomfort. But this does not of necessity point to the part diseased; the pain may be a manifestation of abnormal activity on the part of healthy organs, to which they have been excited by a morbid process elsewhere. In the earlier stages of inflammation of the appendix or with affections of the gall-bladder, the pain may be due to abnormal movements of the stomach and intestine; under such conditions it expresses the response of normal parts to a lesion situated in some allied physiological system.

Now we have reason to believe that the viscera have a double afferent supply ([9], p. 64). One of these corresponds to that deep system, which in the limbs supplies muscles, tendons and joints, and remains intact, when all cutaneous nerves are severed ([9], p. 246). This gives rise to Sherrington's proprioceptive reflexes, that have so profound an influence on the regulation of posture and movement. The peculiar aptitude possessed by a part innervated from this deep afferent mechanism, is the power of responding to stimuli, which produce alterations in form or pressure.* Excessive tension may evoke pain, and "cramp" is the response of these end-organs to abnormal muscular contractions. Parts innervated in this manner are insensitive to light contacts, pricking, heat and cold; but they are so endowed with definite local signature, and the patient is capable of appreciating within certain limits the site of the stimulated spot. It is this system which is responsible, not only for the muscular pains of intestinal colic, but also for the local pain and tenderness on pressure, associated with inflammation of the serous membranes, such as the pericardium, pleura and peritoneum. In fact, these cavities behave like the inner surface of joints ([4], p. 93 and [6], pp. 157 and 232).

But the internal organs are also supplied from another neural system, equivalent, we believe, to the cutaneous punctate mechanism. Even in the skin, the pain-spots are far more numerous than those for heat and cold; protopathic sensibility is essentially a warning mechanism, and most of the viscera, excepting the œsophagus and bladder, seem to be devoid of all sensory response to purely thermal stimulation, as might have been expected from their sheltered position.

One of the most striking characters of pain, or indeed of any sensation, evoked from my hand in a protopathic condition, was its tendency to be referred into remote parts. Portions of the skin seemed to be linked up together in a peculiar manner, so that a stimulus applied to the one produced a diffuse outburst localised in the other. When pain of this order has been aroused in the visceral system, the sensation may be referred to the surface of the body, which becomes tender to cutaneous stimulation. This is not a "hyperalgesia," but a tendency to react more vividly to any excitation capable of evoking discomfort. The threshold to pricking is not lowered, but the vehemence of the response, both sensory and reflex, is greatly increased.

Now this over-reaction is associated with reflexes which still reveal their old impulsive character. The abdominal muscles become tonically contracted, and the leg is drawn up on the affected side. The sufferer is forced to creep into some sheltered place, and to lie curled up until he either dies or recovers. All capacity to move the body as a whole is abolished; the reactions of the part affected now dominate the activity of the complete animal. Visceral reflexes of this order are antagonistic to general movements, and this is expressed not only in muscular immobility but in the mental attitude which accompanies visceral pain ([7], p. 373).

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* Afferent impulses, due to changes in the volume of the lungs, belong to this deep or proprioceptive group [3]. They give rise to the Hering-Breuer reflex; inflation produces an expiratory effect, whilst diminution in volume acts as an inspiratory stimulus. I was able to show that this did not depend on the absolute, but on the relative size of the lung; thus the same volume might produce either an inspiratory or an expiratory effect, according to whether it was reached by allowing the lung to collapse or by increasing its content. Moreover, such stimuli evoke a strong negative after-action in the respiratory centre, so that long continuance of one form of stimulation favours response to its opponent; they exhibit, in fact, a remarkable example of biphasic activity.

Referred pain of visceral origin and the tenderness which accompanies it may be confined to the territory of a few segments only, representing the nerve supply of the affected organ. But if the stimulus is extremely severe, as, for example, during an attack of gall-stone or renal colic, the pain may spread widely even in otherwise normal persons. Not only does it become bilateral, but it is liable to extend both upwards and downwards into regions that stand in no direct nervous relation with the affected organ ([4], p. 104).

Should a referred pain become chronic, this forms an even more important cause for its diffusion. It tends to spread by the fact of its duration. For not only is the general resistance of the patient worn down by the disease, but the passage of painful impulses is facilitated by time.

Certain segmental areas seem to be related to one another, although they are situated far apart on the body. Organs such as the uterus and the breast stand in a close mutual connexion, and within twelve hours of labour, referred pains not uncommonly appear in the front of the chest and in the scapular regions; these come and go with the "after pains," and can be increased by the action of ergot or by manipulating the uterus.

These phenomena of "generalisation" or "spread" of visceral pain and tenderness are of such common occurrence that they form an important factor in the clinical picture of many diseases ([4], p. 105). Diffusion of painful sensations is due to diminished central resistance; potentially painful impulses are allowed to spread widely, which would normally have been inhibited or strictly confined to areas appropriate to the organ affected.

The general bodily state associated with menstruation forms one of the most potent causes of diminished automatic control. This physiological act may be accompanied by referred pain, confined strictly to those segments which stand in direct relation with the pelvic organs; or the morbid sensations may occupy the whole of the body and lower extremities below the level of the umbilicus, with or without the cervical areas and occipital region of the scalp. Finally, the head, trunk, and even the limbs may become painful and tender in parts that have no direct relation to stimuli within the pelvic organs. The extent to which such widespread generalisation occurs, depends more on the temperamental condition of the patient than on the intensity of the painful irritation.

A sudden rise of temperature is also a frequent cause of "generalisation" of pain. Follicular tonsillitis or any parenchymatous affection of the tonsils may be directly associated with superficial tenderness on the same side, extending from the lower part of the mastoid almost to the middle line in front: the so-called "hyoid" area. Suppose, however, that the onset is sudden and is associated with fever, we may now discover pain and tenderness on both sides of the neck, over the forehead, occipital region, and even in the back or other parts of the body and limbs.

Anæmia is another fruitful cause of "generalisation." A patient may be profoundly anæmic, and yet suffer from no pain or tenderness. But, as soon as some source for pain is present, it at once begins to spread. Toothache in an anæmic patient is a diffuse affection, extending far beyond the segmental areas connected in any way with the irritated organ. If the local cause is removed, the pain does not cease, at any rate immediately: for when central resistance has once broken down, control is recovered with difficulty.

In all such cases, the pain originated in some definite and recognisable cause; it spreads widely because of the severity or long duration of the stimulus, or in consequence of the relaxation of central control, which may accompany menstruation, pyrexia, or anæmia. But sometimes pain and tenderness of this order may become widely diffused in consequence of some debilitating psychical state, such as anxiety or emotional shock. Neuralgia and superficial tenderness of dental origin not uncommonly assume undue proportions owing to insomnia and worry, and the mental state of the patient has a profound influence over pains originating in the pelvic viscera.

Occasionally the central resistance to potentially disagreeable impulses is temperamentally so low that pain may appear without any obvious cause for peripheral irritation. Some women are rarely free from tender spots in various parts of the body, representing the maxima of one or more of these tender areas; they form foci from which pain may generalise widely under the influence of emotion or other conditions which lessen the dominant power of higher centres. No gross visceral stimulus is required; the central apparatus is already in an explosive condition, and any relaxation of physiological control leads to an outburst of energy manifested in widespread discomfort. In such cases the order in which the phenomena appear does not follow the rules that can be laid down for pain of visceral origin. Severe pain over the lower part of the abdomen and back may be accompanied by headache and tenderness situated over the forehead; the trunk can be widely affected without the head and neck or *vice versâ*. Such want of harmonious correlation points to the central origin of these morbid phenomena; they are not the direct expression, however extensive, of peripheral stimulation.

Pain and discomfort are the motive force of most elementary reflexes; any stimulus capable of exciting disagreeable effects is naturally shunned, and the organism tends to turn towards those which are pleasurable. But such protective reactions permit of little choice and they must be controlled on the physiological level in favour of more discriminative responses; for without such re-adjustment functional development would be impossible. Moreover, in order to acquire some new facility, it may be necessary to inhibit even normal sensory responses, which, from their disagreeable nature, are intended to compel us to avoid the conditions that evoke them.

For instance, all healthy persons can be made giddy, provided the rotatory movement is sufficiently violent, especially if the head is held in some unusual position. This reaction, even when it culminates in vomiting, is not a pathological condition; it is the normal response to an acute disturbance of equilibrium. A similar vertigo can sometimes be produced by visual impressions, without any change in bodily posture. But the ease with which these unpleasant states can be evoked differs within wide limits in individual cases and varies greatly at different times. Some, who in childhood were unable to swing without discomfort and were sick on a train-journey, cease to suffer in adult life, and, provided the stimulus is not unusually severe, differ little in their reactions from their fellows.

Even in the fully developed nervous system of man, the acquisition of some new aptitude is associated with increasing control over sensory impressions; we learn to adapt ourselves to the conditions around us, and cease to be the victims of uncoordinated reactions. This is particularly evident when learning to fly, for many normal men become giddy, and even vomit on exposure to rapid changes of posture in the air; but the majority quickly recover, and may become expert and bold aviators. They have adapted themselves to the new conditions; the stimuli from the peripheral end-organs no longer dominate the field of response. They are controlled or suppressed, not by the will, but by that power of adaptation which is one of the most potent automatic factors in the activity of the central nervous system ([8], p. 13).

Anything which diminishes this physiological efficiency tends to lessen control over the reaction of the lower centres to sensory stimulation. A gastro-intestinal attack, influenza, an aeroplane accident, domestic worry, or fear, will all act in the same direction. A man, who was giddy when he first went into the air but recovered completely, may fall back to his original mode of reaction, and become unable to carry out any aerobatic evolution, whilst one who has never experienced any previous discomfort may develop undue susceptibility to rotatory stimuli. Such persons are no longer normal, not because the response is a pathological one, but because for a time, at any rate, they have been reduced to a lower level of functional efficiency.

If we responded constantly and inevitably to all impulses from peripheral end-organs, we should be automata, the victims of indiscriminate reactions. But, with the gradual evolution of its functions, the central nervous system acquires ever increasing control over afferent impressions produced by the action of external forces. Such development takes place not only in the race, but in the life of the individual; and it is to this that we owe our power of acquiring new reactions and facilities.

During this evolution of function, the power of responding in a more primitive manner is not lost. The mechanism of the older and more immediate reaction remains intact; but its activity is held in check, ready to become manifest should the nocuous stimulus become unduly intense. All normal persons become giddy if a rotatory stimulus is sufficiently severe, or if it comes into action suddenly, especially if it is greater in one ear than in the other. In the same way, everyone, however healthy, is liable to suffer from pain during the passage of a renal calculus.

But in states of disease this automatic control is likely to break down, less from the violence of the stimulus than from causes which lower physiological vitality. Of these, infective and toxæmic states are often potent on the physical side. Psychical conditions, especially those associated with neural exhaustion, whether temperamental or acquired, may also lead to profound loss of capacity to dominate impulses derived from peripheral stimulation.

Conclusions.

(1) Hughlings Jackson laid down the rule that destructive lesions do not cause positive effects, but induce a negative condition, which permits positive symptoms to appear. I have attempted to apply this law to a number of cases where organic destruction of some part of the nervous system is followed, not only by loss of function, but by some form of over-activity.

(2) Such abnormal reactions cannot be attributed to "irritation." It must not be supposed that we deny the occurrence of true irritative phenomena. These can be studied most easily in cases of injury to peripheral nerves, where they form an instructive contrast to the manifestations of protopathic sensibility. The pain and tenderness, due to definite irritation of nerve-fibres, occupy the whole territory supplied by the affected trunk; but a comparatively small number of the conditions which follow a destructive

lesion can be attributed to this cause. On the other hand, protopathic sensibility is extremely common and occurs solely over parts where finer sensory discrimination is defective. Trotter [19] has put forward the theory that these peculiarities of response are due to exposure of the constituent elements of the closed nervous system to the irritative effect of contact with somatic tissues. Even this view will not explain the abnormally vivid pleasure which may constitute one of the factors in protopathic over-response. It does not help us to understand the peculiar form assumed by the sensibility of the normal glans penis. Nor can it account for the transitory regression and recovery in the sensory condition of my hand during the second stage of recovery after exposure to the injurious effects of cold. The vividness of response and reference into remote parts returned; but they disappeared again when the sensibility of the hand was restored by warmth.

No one has attempted to apply this conception of the irritative effect of contact with somatic tissues to the phenomena of thalamic over-reaction or to other analogous conditions. On the other hand, we have tried to explain such manifestations of over-activity, whether motor or sensory, due to a purely destructive lesion of the nervous system, by the general law of release of subordinate functions.

(3) The conception of levels of activity within the nervous system was one of the fundamental aspects of Hughlings Jackson's teaching. To him a "level" was always one of function rather than of structure. So, whenever I have spoken of higher and lower centres, these must be considered as nodal points of neural activity, and not of necessity as anatomical structures. In the illustrations, chosen to support my thesis, they were anatomically separable; for otherwise it would have been impossible to prove my contention, that removal of a dominant activity, was responsible for the phenomena under investigation.

(4) Removal of a dominant neural mechanism permits the activity of lower centres to appear. These unfettered manifestations are not fortuitous pathological states, but represent that part of a complex reaction, which still remains active; they display characters more primitive than those of the complete functions in which they normally play a part. The response is massive and of unusual extent and vigour; on the motor side it may affect organs which do not normally lie within its reflex arc. Such stereotyped reactions can be excited indifferently from an unusually wide field. They are impulsive and assume the form of a warning or defensive response, regulated more definitely by the extent than by the degree of intensity of the stimulus.

But release from control due to a destructive lesion does not reveal such lower activities in all their original simplicity; for in most cases they have been profoundly modified by the advent of the new centres, which utilise and develop some of the functions originally possessed by the older mechanism.

(5) This dominance of higher over lower forms of neural activity is the consequence of integration within the central nervous system; it is carried out partly by qualitative selection, but to an even greater extent by the struggle for expression between incompatible physiological reactions. This involves permanent inhibition, under normal conditions, of one group of processes, or temporary suppression of these primitive activities until the need should arise for a more impulsive mode of response.

(6) All newly acquired abilities depend on a fresh adjustment of co-ordinated reflexes, and even certain responses from normal sense-organs may be suppressed or held in check. This demands the exercise of permanent physiological control, which may break down under certain conditions, and permit the dominated function to express itself unchecked. A stimulus of excessive vehemence or prolonged duration, any abnormal state which lowers the vitality of the nervous system, and even some inherent want of resistance may all lead to unexpectedly disagreeable and impulsive reactions.

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Preliminary Report of the Mackie Ethnological Expedition to Central Africa.

By the Rev. JOHN ROSCOE.

(Communicated by the Mackie Anthropological Expedition Committee. Received February 15, 1921.)

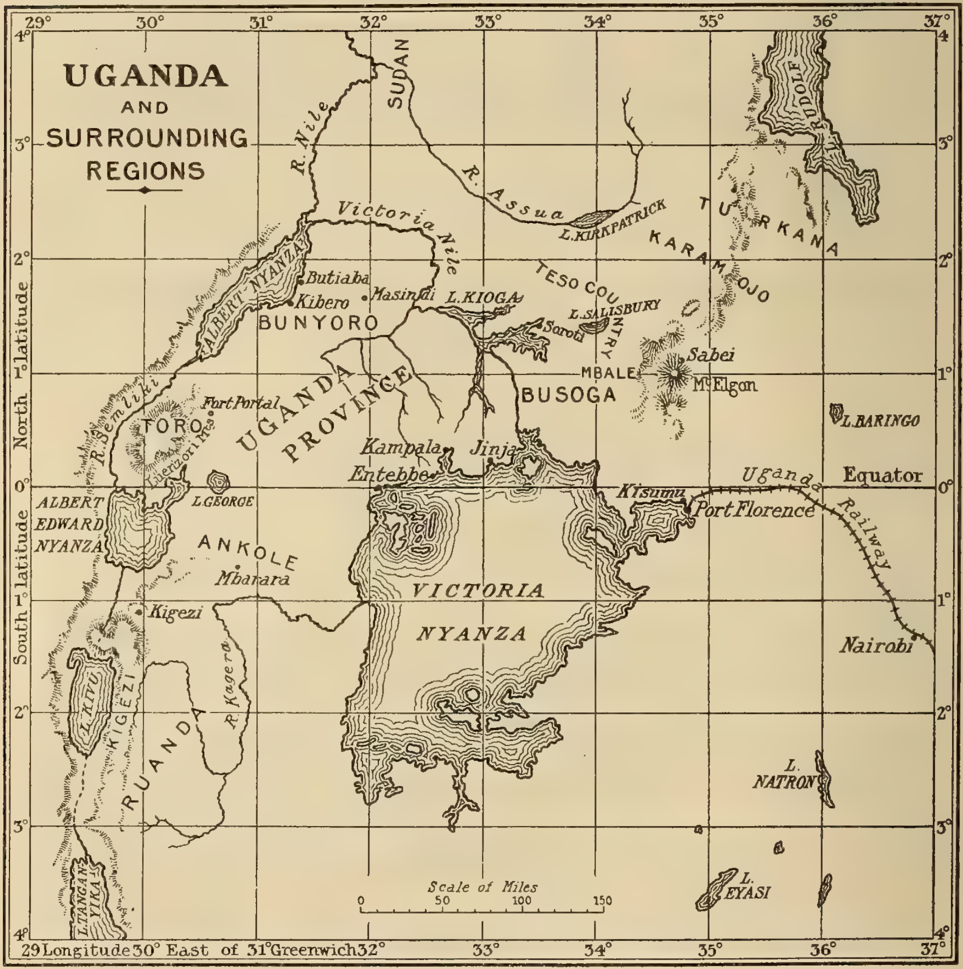
[PLATES 12–16.]

At the outset I realised it would be impossible to find the right men in England to join the expedition, owing, firstly, to the fact that the men whom I should have liked to accompany me were only then returning from France, and secondly, because of the expense. I therefore set out alone, hoping to find at least a photographer and a typist either in Mombasa or in Uganda.

At Mombasa it was impossible to find any man suitable for my purposes, so I journeyed on to Nairobi and spent a week there seeking men; but here again failure forced me to go on to Uganda. In Uganda I thought I was securing the men I required, but I soon discovered they were not suited to my purpose and had to be dismissed.

In Uganda a month was spent at Kampala making enquiries into some of my past work and correcting some things in my former book on the Baganda, There were also men to be interviewed and engaged; but, over and above these matters, I was entrusted by the Government with the task of looking into some native questions which called for attention. I was engaged investigating native secret societies during the whole of the expedition, and it was only at quite the end of the time that I was able to give a satisfactory account of them to the Government authorities.

From Kampala the expedition went in a westerly direction to Ankole (see the outline map), where it was purposed to make the first researches. It had been my intention to return to Kampala from each district, work up the materials there, and send them home. I found this was not feasible, and therefore decided when in Ankole not to return to Kampala again, except for a brief visit to see the Governor.


UGANDA
AND
SURROUNDING
REGIONS
29° 30° 31° 32° 33° 34° 35° 36° 37°
4° 3° 2° 1° 0° 1° 2° 3° 4°
North latitude
South latitude
29 Longitude 30° East of 31° Greenwich 32°
SUDAN
R. Nile
R. Assua
L. KIRKPATRICK
TURKANA
L. RUDOLF
KARAMOJO
Victoria Nile
ALBERT NYANZA
Butiaba
Kibero
Masindi
L. KIOGA
TESO COUNTRY
L. SALISBURY
Soroti
BUNYORO
Sabei
MBALE
Mt. Elgon
BUSOGA
L. BARINGO
R. Semliki
Fort Portal
TORO
Ruwenzori
UGANDA PROVINCE
Kampala
Jinja
Entebbe
Uganda Railway
Equator
Kisumu
Port Florence
ALBERT EDWARD NYANZA
L. GEORGE
ANKOLE
Mbarara
VICTORIA
NYANZA
Kigezi
R. Kagera
L. KIVU
KIGEZI
RUANDA
Nairobi
L. NATRON
L. EYASI
L. TANGANYIKA
Scale of Miles
0 50 100 150

In Ankole my attention was devoted to the pastoral tribe known as the Bahuma, which is the descriptive term used in that part of Africa for the pastoral people. I found the Bahuma were of the Hamitic stock, and that they had entered into the country some hundreds of years before and had lost all traces of their original home. They could only say they came from the north-east. They still strictly abstain from intermarriage with any of the tribes who are not of the same stock, and adhere to a milk diet. This tribe contains the purest set of pastoral people in all that part of Central Africa. Numbers of the lower classes in the country districts observe a pure milk diet and abstain from eating any vegetables or any butcher's meat except beef, and after eating beef they abstain from drinking milk for some twelve hours. Here the idea of transmigration was common, the king being supposed at death to become a lion. (Figs. 1 and 2, Plate 12.)

I found that the king's sister, though called the queen, was permitted to marry, and the other princesses married chiefs of their own tribe, though they are careful to find a husband from another clan. This custom differs from that of the other pastoral tribes, where princesses are forbidden to marry. Throughout the country there exists clan exogamy, and descent is reckoned through the male line.

Marriage is chiefly contracted during infancy, the arrangements being made by the parents of the two children. It was strange to find that, though the people are on the whole a monogamous tribe, there are many instances of polygamous marriages, especially if the first wife is sterile. Again, there is the practice of polyandry, owing chiefly to economic conditions. A man who wishes to marry and finds he is unable to provide the necessary amount of milk for himself and wife, will invite one or more of his brothers to join him, and together they will marry a wife. The woman does not object to the multiplicity of husbands. The children born are reckoned as the sons of the eldest brother. Another strange marriage custom is, that when the husband's father visits his son, the son will vacate the house and leave his wife for his father, who takes possession of the house and his daughter-in-law.

There is a firm belief in ghosts, and numbers of cows are devoted to the ghosts of men. The milk from these animals is daily placed before the shrine for the ghost's acceptance, and later the man, with his children, will take a sacred meal in the ghost's presence. Thus twice daily there is a communion with the dead.

The customs of the agricultural people in this district were also found to be instructive. These are the aborigines, who are called slaves by the pastoral people. They do the work for their superiors, such as tilling the land, herding goats and sheep, and keep their fowls. Goats and sheep are used for trading purposes to buy salt for the cows and weapons for the men, and fowls are wanted for divination. The customs of the agricultural people differ from those of the pastoral people, though the former seem to have adopted many of the customs of their masters. (Figs. 3 and 4, Plate 13.)

The ethnologist soon learns that in the district, now known as Ankole, there are two or three tribes of the Hamitic stock, who, through the British occupation, have been merged into one nation.

From Ankole the Expedition passed further west to the district known as Kigezi, which stretches to the Belgian Congo on the western side, and is bounded by Ruanda on its southern side. This is a mountainous country, occupied by several tribes. The most populous tribe is called Bakyiga. These are agricultural: they cultivate the sides of the mountains and also possess large herds of cows. They are a wild people, strong and fearless. They have never submitted to the pastoral people, though I found the pygmies had driven them back from one part of their country bordering on the Congo. The mountains in this district are very fine, and there is an active volcano, known to the inhabitants as Bufumbiro, which means Cookhouse.

While studying the Bakyiga one was struck by the similarity of their customs to those of the Bagesu on Mount Elgon. It was impossible to obtain any information as to their past history: they stated they knew nothing of themselves beyond their grandfathers' time. They have no over-chief, and no method of recording events could be discovered. I found they buried their dead near their huts, and there is no trace of cannibalism. In this they differ from the Bagesu, who are ceremonial cannibals and eat their dead. The disregard for life is very marked, and the clans are so hostile that it is unsafe for a man to leave his home and walk a mile into another district alone: he would be speared to death when he crossed the border.

When a man wishes to marry, he asks one or two friends to help him to find some young woman engaged in cultivation; the party then lie in ambush until she is alone, when they kidnap her and march her off to their home. From a hill they call to the girl's father, and tell him to come and take the dowry for his daughter, who is now to be the wife of such a one. Should the brothers of this woman meet a brother-in-law, they do not hesitate to murder him. There is no bond of relationship through marriage. Deeds of violence are common; I saw an instance during the few days I spent in the district. A man wounded a clan brother of the same village, who wanted to sell him a goat for a higher price than he wished to give. The purchaser marked his disapproval by thrusting his spear through the thigh of the seller of the animal.

I also heard that murder was quite common, even though the punishment is severe. Two murders were said to have taken place a few days before my arrival. In each case the murderer, after being bound hand and foot, was tried, and, when proved to be guilty, was buried alive under the corpse. When I reached Mount Elgon, some months later, and was making enquiries among the various clans of Bagesu, I found a clan calling themselves

Bakyiga, and was informed by them that a part of their clan had migrated and gone south, and had not since been heard of. In all probability, the Kigezi tribe of Bakyiga is the missing clan, who have modified some of their customs.

Passing along the eastern side of Lake Edward, the expedition met with numbers of pastoral people, some of them with very large herds, which had not then been affected by rinderpest, which was devastating Ankole. When nearing the river which connects Lakes George and Edward, a tribe of Baganda was met with. These men were obliged to leave Buganda, because their chief had killed a prince in battle. At the time the deed was done, his action was highly commended by the king, but later the priest warned the king that, unless he put the man to death, the ghost of the prince would cause disease in his country. The chief heard that he was to be captured, so he fled with a number of people, and, passing through Ankole, settled near Lake Edward. There he and his relatives, with some followers, live to this day. They have adopted many customs of the surrounding tribes, with whom they intermarry.

Crossing the river we entered Toro, and I was able to see a little of the cannibal tribes of the Luenzori Range, though I only remained at the capital a week, while waiting for a steamer to carry me over Lake Albert into Bunyoro. The cannibal tribes are of the degraded kind, who will kill and eat men of other tribes.

The pastoral people of Toro are an offshoot of the Banyoro, and on this account there was no necessity for staying among them to learn their customs; I could do it better among the Banyoro. I therefore passed over Lake Albert and spent four months in Bunyoro. In this part of the Uganda Protectorate I did some of the best research work. The king was anxious to help forward the cause, and took great trouble to secure for me the best informants. Not only did he procure these for me, but he gave a week's pageant of the old milk customs, when from morning until night were enacted the old ceremonies. After this I was with him a month, spending many hours each day while he explained what I had seen, and gave information which he had obtained from other old people, some of whom were present with us during these interviews.

The king in the past was the high priest, or perhaps it might be said that he was the Deity, for so he was regarded by the common people. Each morning, before he rose from his bed, a girl, who had to sleep across the foot of the bed, lest the king's feet should come against the bed frame or be exposed, had to anoint each great toe. After this the king went into a yard near the throne room, where two yearling bulls, one a black animal with a white spot on its forehead, the other a red and black animal with a white spot on its forehead, were driven to him. The king took the black bull by the horns and uttered a few words, laying upon it any evil of the night; he then took the red and black bull in the same manner, and uttered a few words of blessing on the dawning day. After this he performed his cleansing ceremonies amid fetishes: he next went to see the sacred cows milked. The men and maids who performed this duty had to be ceremonially clean for two days before they entered upon their office; faces, arms, and chests were whitened with clay. So solemn were the duties, that they were able to carry them on for two days only, when they were relieved by another set of milkmen and maids.

When the sacred milk was ready for the king, another dairy-maid announced the fact, and the king rose from his throne, and withdrew into the dairy to drink. When he rose from his throne, the guard at the door proclaimed the fact that the king was going to drink milk; and men and women, within the royal enclosure, knelt and covered their faces. They might not cough nor clear their throats during the time the king was drinking milk, on pain of death. When he returned, the people rose and went about their usual avocations. (See Figs. 5–8, Plates 14, 15.)

A similar custom was observed when the king took his meal in the afternoon. Then the royal cook, who, like the milkmaids, was purified, and had his face, chest, and arms whitened, came into the king's presence, with a pot containing beef from an animal of the sacred herd. The beef was cooked and cut into small pieces ready to be eaten. The cook took a two-pronged fork, which he held in his hand, knelt down before the king, dug the fork into a piece of meat, and put it into the king's mouth. Four times he did this; and, should he by accident touch the king's teeth with the metal, he was put to death on the spot. In the evening a herald announced that the sacred cows were coming, and people fled on all sides, and covered their faces until the animals had passed. The king sat while two animals were milked, and again drank the ceremonial milk. At other times during the day the king might drink milk without ceremony, but he must not eat meat.

In later days, when the kings began to eat other food, they did so by stealth; they took it standing in a hut in a courtyard, where no outside person could see or know the king was having a meal. The food was cooked outside the royal enclosure, and carried in as though it was intended for the servants, and was kept ready for the king, who would escape from his court and priestly duties for the meal. By night the king prowled round his enclosure with one of his pages until quite late. He slept during the early hours of the morning in the hut of a wife, but always completed his rest in the court house in a royal chamber.

There was a royal guild to which chiefs were admitted by a customary rite of drinking sacred milk; after which they were given a crown to wear. So solemn was this rite, and so much dreaded, that chiefs who were admitted often fainted during the ceremony under the strain. When admitted, they became a trusted body united to the throne by special ties.

When a chief died his death was announced to the king in the following manner; the relatives chose two or three of their number to go with a cow, in the early morning, to the entrance gate of the royal enclosure. The men drove the cow to the gate at a run and shouted the name of the dead person saying "Death has robbed you"; they then turned and fled for their lives, because, near the gate were guards, belonging to the chief who had charge of the king's tombs. The guards charged after the fleeing men and, should they catch one of them, they killed him on the spot. The guard then returned, caught the cow in the roadway, and killed, cooked and ate as much of the animal as they could before the sun rose. Any meat, bones or offal remaining at dawn was buried before the sun shone upon it, and the place was cleaned. The custom when a son is acknowledged to be heir to property is also of interest; the king was apprised and gave his consent to the choice. The man must then appear in the king's presence and go through a form of allegiance, before he enters on his inheritance.

The annual custom when a man, chosen from a particular clan, is sent to personify the late king and to reign in his tomb, is worth noting. The man sits in the tomb as king and has full use of the widows; he distributes royal favours during the week he reigns, and is then taken to the back of the tomb and strangled.

From Bunyoro the expedition went to Soroti, on an arm of Lake Kioga nearest to Karamojo. It was my desire to pass through Karamojo to Turkana, and on to the Galla tribe near Lake Rudolf. In this I was disappointed owing to tribal disturbances. It was considered unsafe to go without an armed escort; and as that would have thwarted ethnological research, I decided to go to Mount Elgon, passing through the Teso country, and keeping south of Lake Salisbury. A few customs were studied among the Bateso and Bagesu, who are naked people, and whom I found to be ceremonial cannibals.

Hearing there were initiation ceremonies being performed by the Bagesu on Mount Elgon, I pushed forward to be in time to see them. In this I was successful, though it required some tact and persuasion to be permitted to see the actual circumcision rites. The details of these customs are harrowing; they call for great fortitude on the part of the candidate. The youths are worked up for days to a high state of nervous resistance. On the day of the ceremony they undergo strenuous exertion, and are fortified for the ordeal by a special meal and native beer. After this they take the oath of tribal allegiance by jumping upon an egg, and then stand as though unconscious of any pain, while the operator saws, with a large knife, the flesh from the whole of the member and casts it under the youth's feet. (Figs. 9, 10, Plate 16.)

These Bagesu do not bury their dead, because they think the ghost will kill the children of the family should they allow the body to decay. The corpse is thrown out after dark, when men go out with trumpets made of gourds and imitate the cry of jackals; meanwhile a number of old women proceed to cut up and carry back the corpse into the hut of mourning. There, during the following three days, the mourners eat the body and wail.

From the Bagesu we proceeded up Mount Elgon, round to the most north-easterly point, to investigate the caves which are said to have been inhabited formerly. These caves are still used from time to time; we visited three of them, but could find no traces of permanent habitation. Report says that, before the British occupation, they were used frequently as places of refuge during any raids made by people from the north. The raiders were evidently Turkana and Abyssinians.

While on Mount Elgon the customs of the Sabei were studied, and I witnessed some of their marriage dances. A discovery was made with regard to the two sets of pastoral peoples, both of them evidently from the same Hamitic stock. Here was the dividing line: the Masai, Turkana, Nandi, and Somali tribes have the rite of circumcision when the youths are initiated, and the women undergo a corresponding rite; whereas the pastoral people in the south will not submit to any such mutilations. These two sets of people are also most hostile the one to the other. The pastoral people of the south seem to be the older immigrants.

On the upper parts of the mountain there was found to be a tribe of trappers living, whose diet is mainly moles and rats and the young shoots of bamboo. They obtain a certain amount of grain by barter with members of other tribes in exchange for their dried mole flesh.

Retracing my steps to Mbale, the Government station for the district, I found, before descending to the lower slopes of the mountain, a tribe who call themselves Bakama. The name interested me, and I set to work to investigate who they were. I found them to be an offshoot of Bunyoro smiths, who had migrated and adopted the customs of the mountain pastorals, with whom they intermarried, thus becoming a separate clan of those people.

The expedition then passed to the east of Mount Elgon, in company with the Provincial Commissioner, and came round to Jinja. While in Busoga it was possible to make notes on the Basoga, and to work my way back to Lake Kioga. From the most easterly point of the lake I took a steamer and reached Bunyoro again, to commence the journey down the Nile.

During this time I had been fortunate enough to find someone in each tribe who knew one of the languages I spoke, and I could thus obtain my information at first hand.

We crossed to the north of Lake Albert to Nimule, and journeyed 120 miles on foot to Rejaf, where I discharged my boys, and came on to Khartoum alone.

At Rejaf I had the great advantage of being with the Rev. A. Shaw, of Malek, who is conversant with the tribes contiguous to the Nile; and through him I learned much that was useful as we passed down the river. The information thus gained inclines me to think that the various Sudan pastoral tribes, commonly called Nilotic, are closely related to the pastoral tribes of Central Africa. Here and there I saw, or learned of, customs which I had also met with among the Bantu pastorals. It is important to record the tribal customs of these Nilotics before they become contaminated by western ideas or Muhammedanism.

From Khartoum I went to Cairo and thence returned home.

The expedition was undertaken chiefly to study the pastoral tribes of Ankole, Bunyoro, Karamojo and the Gallas. It was hoped to obtain details of the social anthropology which would be of value to science and to the Government, especially in regard to customs affecting land tenure, inheritance, marriage and birth.

It was realised that the natives would benefit if such customs could be reduced to writing and made available for Administrative Officers and Missionaries. I departed from England on June 14, 1919, and returned on November 15, 1920, so the work may be said to have occupied seventeen months.

It will not, I hope, be out of place to put on record the debt owing to Sir James Frazer, through whose untiring energies interest was aroused which resulted in the expedition, and to thank Sir Peter Mackie, who not only provided the funds necessary for the expedition, but also relieved me of much of the preliminary labour of organisation. His active help was also given throughout the months I was in Africa.

Mr. Wellcome was good enough to supply me with medicines for the expedition. These I found of great value, both for myself and for my men.

Of those who helped me in Africa I should like to thank the Governor of Uganda and his staff for their kind and courteous help, and for the many tokens of personal interest I received at their hands.

DESCRIPTION OF PLATES.

PLATE 12.

Fig. 1.—The king of Ankole, who is noted for his height, being about 6 feet 7 inches. The staff or sceptre he holds was handed to him each morning; he first put it to his forehead and then to each shoulder to destroy any magic or other ills of the night.

Fig. 2.—Ankole royal drums. Drums were not used in Ankole. These drums, said to have been brought by an early king, were held to possess spirits. They were kept in a temple and had a herd of cows devoted to them. Votive offerings were made to them of cattle. No cow might be killed or removed unless the spirits consented. The sanction was obtained by the priest by augury. Each morning and evening milk was offered to the drum spirits, and after it had been some time before the drums, and the spirits were supposed to have taken the essence, the priests drank the milk in the temple.

PLATE 13.

Fig. 3.—Medicine-men exorcising a ghost. These men laid the patient on his bed, and, after using incantations over him, they made incisions on his chest and forehead, and rubbed in powdered herbs. One of them chewed certain herbs and expectorated the juice into the mouth of the patient, while the other man sang incantations and used his rattle over him.

Fig. 4.—Pots used as drums for dances. The water-pots are beaten on the mouths by flat leather pads, the different sounds being obtained by various amounts of water being poured into each pot.

PLATE 14.

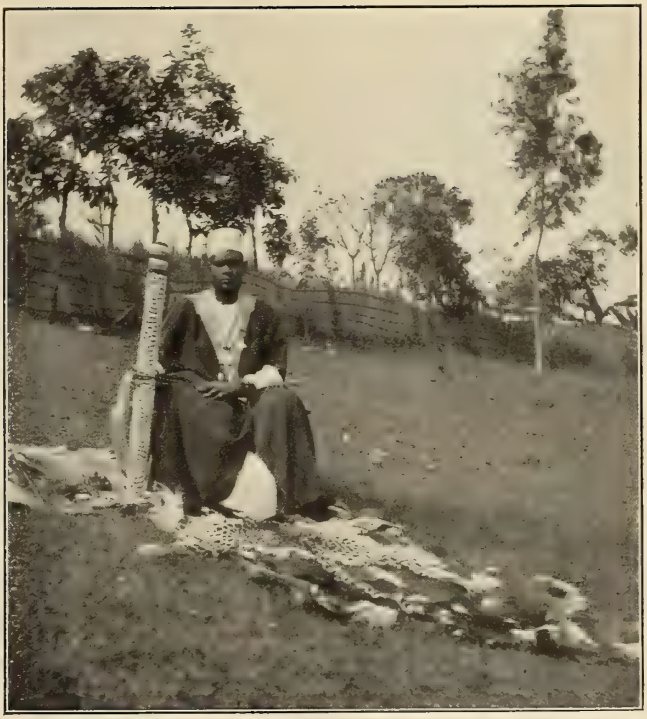
Fig. 5.—Bunyoro milk customs. The king had a herd of nine sacred cows; two were milked for his special use, the others were wanted for milk for ceremonial uses, and also to give to favourite wives. The milk was kept in the dairy, where the king went at stated times to drink it. The men and women engaged in this work were purified for their office.

Fig. 6.—Bunyoro. When the king retired to drink the sacred milk everybody in the royal enclosure knelt and covered their faces. Should any sound be heard, especially that of clearing the throat, the culprit was put to death. When the milkmaid handed the pot of milk to the king she screened her eyes with the cover of the pot, while with her other hand she used a fly-brush, waving it to keep off insects.

PLATE 15.

Fig. 7.—Bunyoro. Type of cowman who milks the sacred cows; during the time of his office he may not see or speak with ordinary men from outside the kraal; during the milking he must avert his eyes to avoid seeing the milkmaids.

Fig. 8.—Bunyoro new moon dance. With each new moon there is a festival lasting nine days. During these ceremonial dances some solemn rites are performed, such as admitting members to the sacred guild, trying members of that body who have offended, and sentencing them to death or acquitting them.



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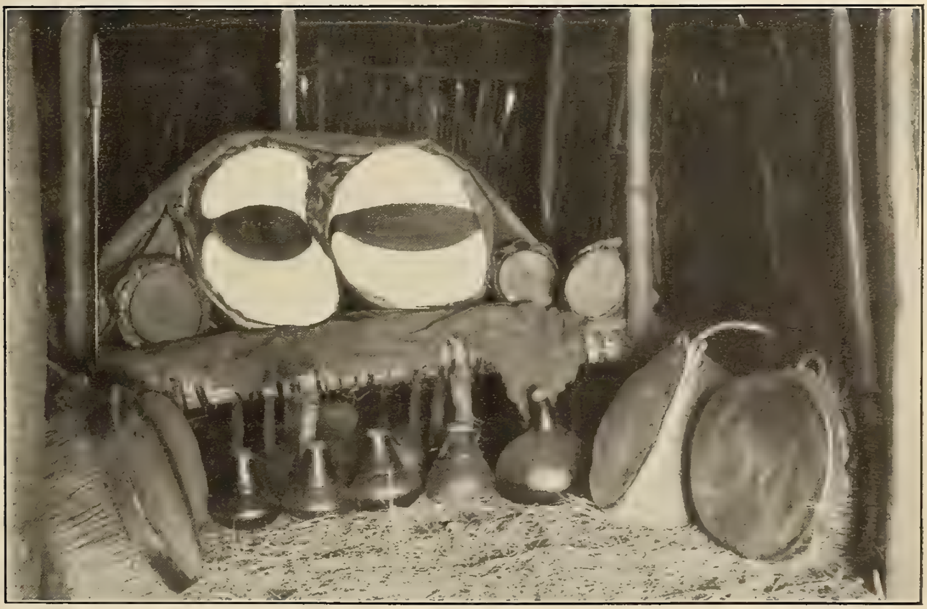

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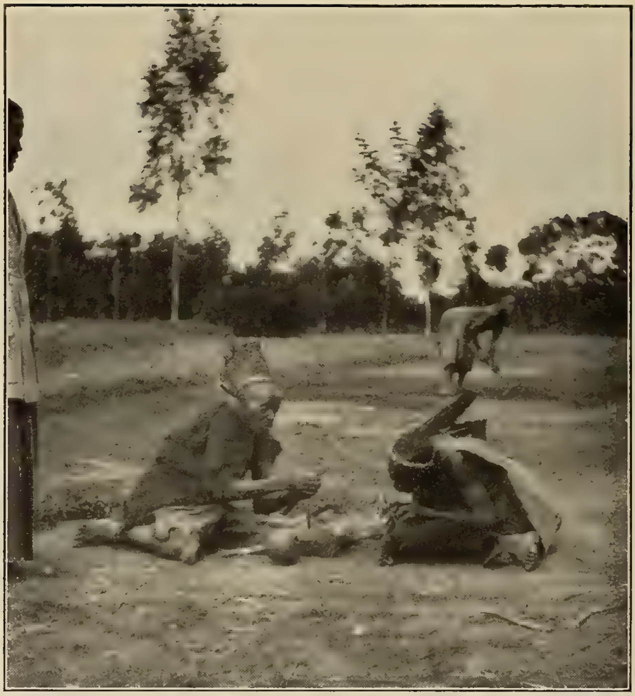

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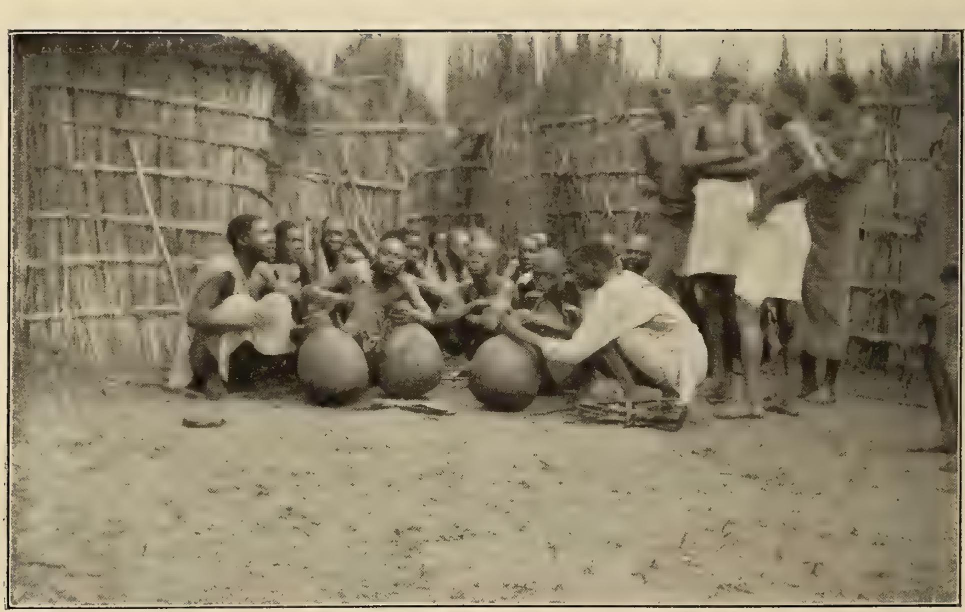

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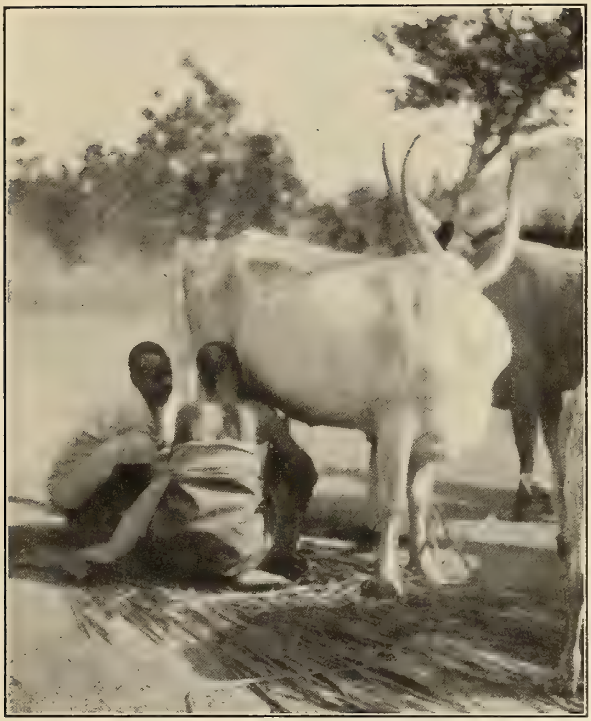

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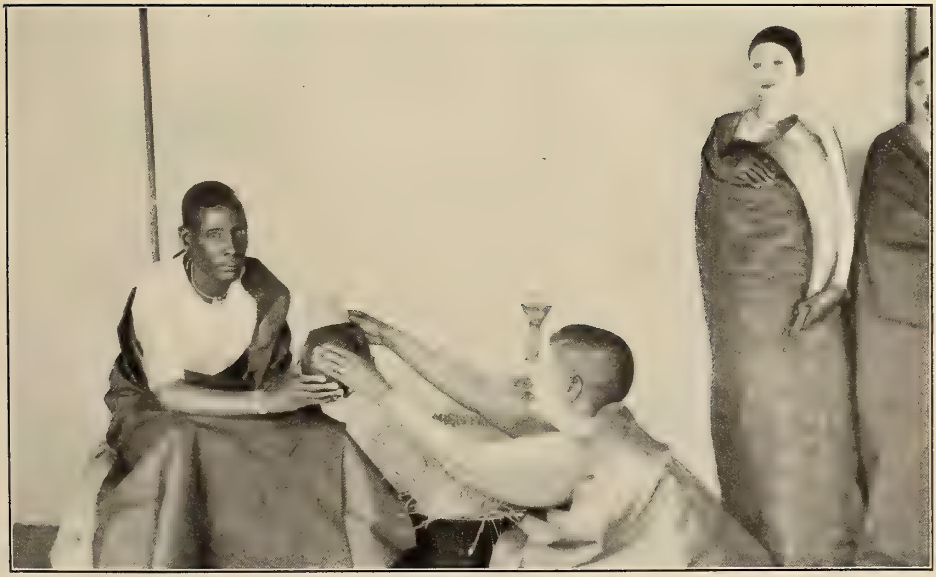

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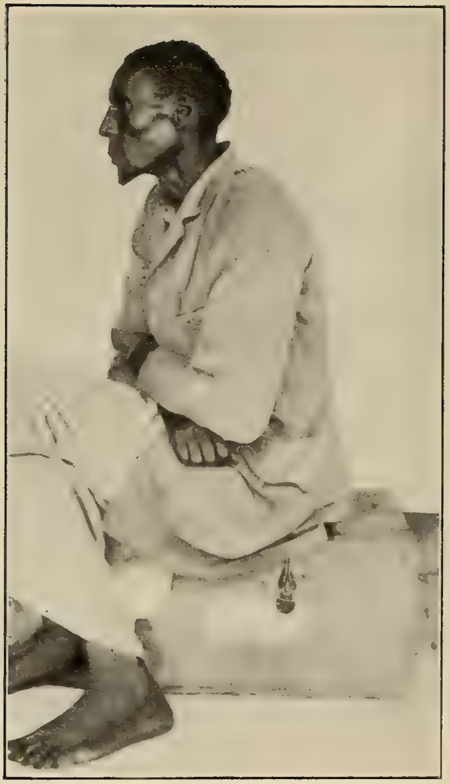

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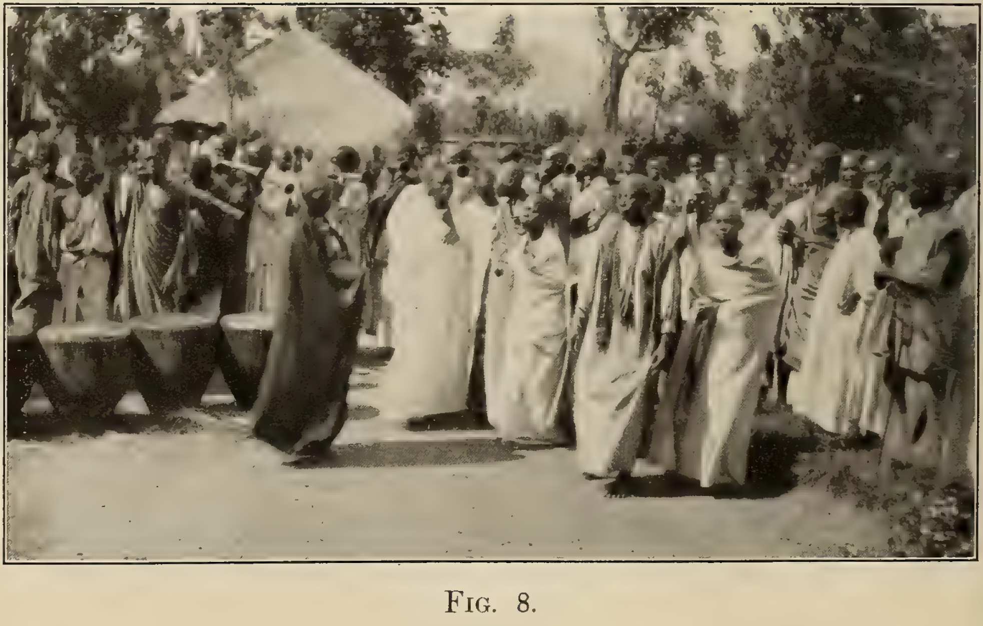

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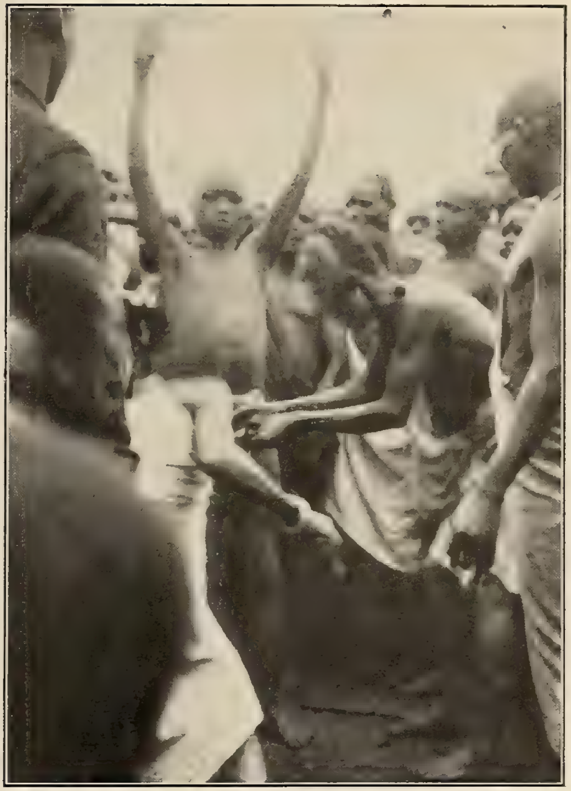

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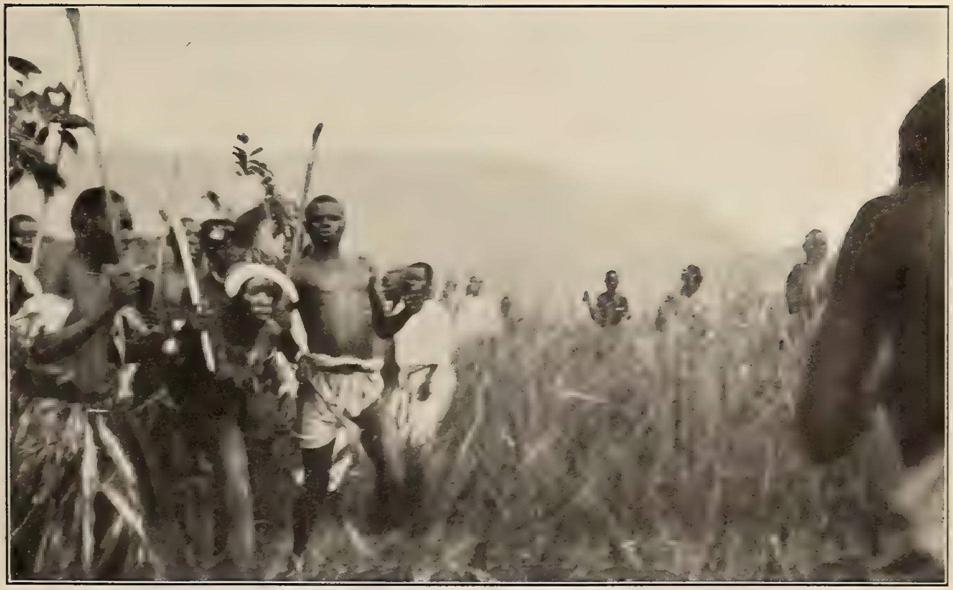

FIG. 9.

PROCEEDINGS
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Plate 16.

Fig. 9.—Mount Elgon Initiation Rites. Before the initiation ceremony the young men perform dances at various villages and receive gifts for the circumcision feast. On the day of the ceremony, before the event takes place, they go round the village and drive out hostile ghosts supposed to be lurking round.

Fig. 10.—Mount Elgon. During the operation of circumcision the patient stands with arms extended upwards, and must show no sign of timidity. Should he either utter a sound of pain or move a muscle he is branded a coward. Shame will force such a youth to commit suicide rather than face his companions afterwards.

A Quantum Theory of Colour Vision.

By J. Joly, Sc.D., F.R.S.

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In a paper on the subject of the Quantum Theory of Vision, issued in the 'Philosophical Magazine' (February, 1921), I dwelt on the view that the sensation of light is in every case stimulated by the action of photo-electrons set free in the retina. Further, the energy of the photo-electron being proportional to the frequency of the light, the strength of the stimulus produced is the all-sufficient origin of colour sensations. That *colour* is entirely a cerebral phenomenon is evident. Light, visible and invisible, consists of a uniformly graduated series of wave motions or energies. There is nothing to distinguish one part of the spectrum from another save the difference of wave-length or frequency. But objects in Nature react differently towards these waves: absorbing some, reflecting others, and so the selective effect of natural objects towards light has discovered to the organism a means of improving on mono-chromatic vision: a means of distinguishing objects by their selective absorption and reflection. Our colour sensations were developed solely for this purpose and solely under the influence of the light reflected by natural objects. Hence, a limited number of fundamental sensations being the simplest, if not indeed the only, way of securing the desired end, we would expect that these sensations would be developed so as most effectively to interpret the frequencies met with among natural objects reflecting solar light. The evolutionary development of three highly developed colour sensations according to the extreme and mean regions of the spectrum is the result. Colour sensations, *i.e.* (white), red, green, and blue were evolved, whereby the whole gamut of the spectrum can be dealt with.

In my former paper I restricted my reference to colour vision to this evolutionary aspect of the matter. In the present paper I have something to add respecting the retinal apparatus developed to subserve the end which Nature had in view.

(1) The law defining the stimulation of excitable tissues known as the "All or none" law is generally accepted by physiologists. It comes to this: in order that a disturbance be set up and propagated in a nerve, the exciting cause must possess a certain minimal intensity. The intensity of the transmitted effect is a constant proper to the particular tissue. When end effects—*e.g.*, muscular contraction—exhibit different grades of intensity, this is due to difference in the numbers of fibres activated by the primary stimulus. If one fibre only existed in the nerve, one intensity only of end effect is possible, whatever be the intensity of the exciting stimulus. There may be a summation of stimuli. A stimulus too weak to activate the nerve leaves behind it a local change at the point of application. A second stimulus arriving before the condition induced by the first stimulus has passed away may produce the effect of the minimal stimulus.

The existence of a "refractory period" must also be noted. This appears to be a period of recuperation during which the nerve fibre regains its excitability. "All excitable tissues are incapable of response to a second stimulus applied at a short interval of time, differing in different tissues, after a previous one."* In the latter part of the refractory period stimuli stronger than normal may secure excitation, but the magnitude of the disturbance propagated is subnormal. The refractory state appears to act as inhibiting the transmission of a too sustained and intense disturbance; the recuperative influence at the nerve terminal being, as it were, used up as fast as it is generated.

The refractory interval must vary greatly according to the nature of the tissue. It may be as great as 0·002 sec. when nerves controlling muscular excitation are concerned. Stimuli travelling over nerves concerned with audition must succeed each other many thousands of times in a second in order that note-frequency be interpreted to the brain. Thus the researches of Wrightson and Keith point to four impulses per wave being necessary. Middle C would, therefore, require over 1,000 impulses per second, and a note of 15,000 vibrations per second (higher and audible) would require 60,000 impulses per second.†

(2) The bearing of these laws on the present subject is obvious. If the

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* Bayliss, 'General Physiology,' p. 433.

† Keith in Sir Thomas Wrightson's 'The Analytical Mechanism of the Middle Ear,' 1918, p. 212.

varying energies of the photo-electrons are to be transmitted to the cerebral cortex, and if such energy differences cannot be conveyed by the nerve fibre, the alternatives presented to us are either to reject the theory altogether or to assume that more than one nerve fibre transmit the stimuli concerned with colour vision, and that it is by the number of fibres stimulated that the energy is interpreted. This naturally sends us to the histology of the retina, because as colour vision appears to be restricted to the cones and achromatic vision to the rods, the connections of these organs with the optic nerve might be expected to reveal an anatomical difference in accordance with the structural features we are driven to assume as existing in the cones.

This anatomical difference is presented to us with remarkable clearness. All delineators of these minute organs agree in depicting the rod as connected through a fibre of extreme tenuity with the deeper lying nervous structures; while on the other hand, the corresponding connection of the cone is, relatively to that of the rod, exceedingly bulky. The accompanying figure (after Greeff) shows these features in a conspicuous manner. Obviously many such fibres as lead from the rod might be contained in the cone fibre. Indeed it is difficult to account for the remarkable difference of structure on any other suggestion.

(3) I assume that the explanation is correct and that the constituent fibres of the cone connection are collected in the spindle-shaped enlargement of the organ and brought to the base of the percipient extremity, where their terminals are activated by energy carried into the cone by photo-electrons. It may be that the fibrils persist into the cone or into the cylindrical percipient extremity of the foveal cone. In either case the number of fibres activated depends on the kinetic energy of the photo-electron.

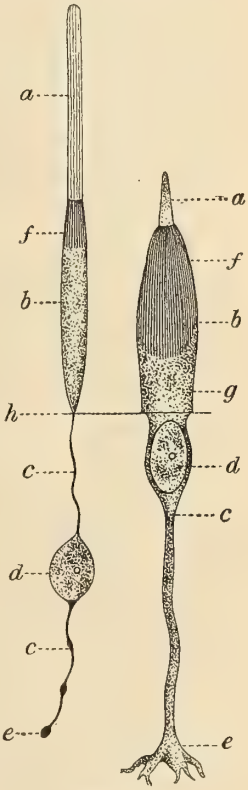

Fig. 1.

On this view we find a purely anatomical reason for the differing functions of rods and cones. The photo-electrons liberated within the rod can transmit but the one intensity of stimulus to the brain, no matter how much they may differ in kinetic energy. Hence its achromatic functions and the low luminous character of scotopic vision. A one-fibre stimulus is all it can send. On the other hand the cone sends a number of stimuli proportional to the energy of the exciting electron; and evokes grades of sensation owing to the possession of more than one fibre connection with the brain.*

(4) It may be objected that the energy of the photo-electron is too small to be divided. The answer is that it is not the energy of the electron which travels to the brain. The "all or none" law and the fact of the refractory period plainly indicate that the stimulus is a trigger action. And where we are dealing with the equilibria of molecular systems of extreme delicacy we are not entitled to limit the possibilities. True we are not in a position to state the character of the operative mechanism. But here, as elsewhere in physiological and physical science, we necessarily leave, and are clearly entitled to leave much to the unknown.

(5) Although the conditions involved appear to be very complex some discussion of the bearing of this view of the structure of the cones on colour vision may be permissible. It is evident that the activation of the nerve fibres in definite numerical groups according to the energy of the photo-electron may be regarded as realising Young's three kinds of nerve fibre or the three "apparatus," which, by various writers have been postulated as accounting for colour vision.

(6) We are presented in the visible spectrum with a series of frequencies ranging over about one octave. The corresponding quanta appear in the photo-electron and must be reckoned with, whatever view we may hold respecting the number of primary colour sensations.

It will, however, conduce to clearness and at the same time, very probably, lead us nearest to the actual facts if we assume three primary colour sensations.

Various spectral positions have been assigned by different authorities to these sensations. The difficulty of the subject is revealed by these differences. Helmholtz bases his selection on curves plotted to represent the rate of change of hue attending change of wave-length. Such curves show two regions of special sensitivity respecting change of hue; one in the yellow and one in the blue-green. These positions of maximum change, although originally derived by direct observations,† mark the intersections of the colour sensation curves and may be referred to parts of the spectrum where the ordinates of the colour curves are most rapidly changing in relative value. Helmholtz's fundamental green sensation is located between these points at 540 $\mu\mu$ to 560 $\mu\mu$. König's green sensation curve attains its maximum height at about this point, and as might be anticipated it is in a region of *minimum* change of hue with change of wave-length. Abney's green sensation is at about 515 $\mu\mu$ its position being based on the colour vision of the green blind.

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* It seems not improbable that the evolutionary development of the cone originated in the fusion of the rods to form a single organ. The rods are known to be anastomosed in groups with a single conducting neuron.

† Parsons' 'Colour Vision,' Cambridge, 1915, p. 30.

The red sensation according to Helmholtz and König lies at the very extremity of the visible spectrum, in the purplish red or a little beyond it. That is at 800 $\mu\mu$ or even greater wave-length. The blue sensation is defined by Helmholtz as an ultramarine blue. Abney takes a point in the violet beyond G. This will be, say, at 420 $\mu\mu$.

It will be found, taking Helmholtz's green as at 550 $\mu\mu$, that the frequencies at these three points may be represented by the three numbers—2 : 3 : 4. The blue and the red sensations are thus an octave apart. To this fact the blue tone of the extreme red and the red tone of the extreme blue have been before now ascribed: the octave in auditory sensations being the most perfect of the concords. The physical interpretation of this will be presently shown.*

(7) According to the present theory, the frequencies just cited as proper to the fundamental colours would bear the interpretation that the "red" quanta (*i.e.*, quanta stimulating the red sensation) act by stimulating two nerve fibres of the cone. The "green" quanta stimulate three fibres; and the "blue" quanta stimulate four fibres of the cone, or numbers of fibres proportional to these figures; although for various reasons the smaller numbers appear to be most probable.

(8) As we know, the several sensations are stimulated by a considerable range of frequencies, and, in fact, they largely over-lap. This condition necessarily arises. It is certain that every quantum of the visible spectrum is competent to activate two fibres, and may do so even if they carry energy sufficient to activate three or four fibres. On the other hand, we may not assume that those quanta which most actively stimulate a fundamental colour sensation represent the minimal stimulus which will activate the particular number of fibres involved; but rather that this number of fibres may be supplied not only by superior quanta but also by quanta from

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* It is possible to find the relative values 3 : 4 : 5 in the frequencies of fundamental sensations. The end-stretches permit a choice of frequencies over lengths where there is no change of hue. Suppose, then, we take for the fundamental green sensation 505 $\mu\mu$. This gives a frequency which we represent by the number 20. The other frequencies taken as 15 and 25 give the wave-length 660 $\mu\mu$ and 400 $\mu\mu$, both of which are within the end-stretches. On these figures, however, the red and violet sensations are not an octave apart. There is evidence that they are, in fact, an octave apart; see (6). These figures, too, involve a larger minimal number of fibres. Here, again, the simpler ratios commend themselves.

neighbouring inferior points on the spectrum. The symmetrical curve of the green sensation perhaps best reveals what is happening, for in the case of the red and blue sensation curves, end conditions imposed by the failing absorption of the visual purple intrude themselves and largely define the external slope of the curves. In the case of the green curve we find a rounded crest of maximum green sensation, and in the slopes leading up to it we perceive the increasing number of quanta which go to activate three fibres and thus create in our cerebrum the green colour sensation. At the same time we see by the over-lap of the red curve that this region also pays toll to the red sensation; many quanta degrading to stimulate but two fibres.

In those cases where stimulation is attended by a misfit there may also be one-fibre stimuli. The one-fibre stimulus simply creates the luminous achromatic sensation proper to the rods.

The stimulation of the red sensation—*i.e.*, of two fibres—is displayed the whole length of the spectrum. It falls off rapidly after the demand of the three-fibre sensation begins to make itself felt, but continues right into the violet. According to Abney's measurements, the violet sensation includes 72 per cent. of red and 28 per cent. of blue sensation. Here the value of the quantum has risen to fully twice that required for a two-fibre stimulus, and in this way, possibly, excites in the cerebrum the sensation of red.

The blue colour sensation curve—according to König—gives away relatively few quanta to the green sensation curve. This may be explained, perhaps, in this way. The stimulation of four fibres leaves five vacant fibres. If a second stimulus of four fibres takes effect there is not room for a three-fibre stimulus. Now in the case of the "green" quanta spending themselves on two-fibre stimuli the element of chance is more in favour of the degradation. Two successive three-fibre stimuli leave room for a two-fibre stimulus.

(9) On the present theory the points of maximum change of hue for a given displacement along the spectrum is physically explained as follows:—Where colour sensation curves over-lap we know that quanta are being distributed among the fibres in two or three different ways. Thus, for instance, some may go to create two-fibre stimuli and some to create three-fibre stimuli. At some point on the spectrum, where the two-colour curves intersect, an equal number of quanta stimulate each sensation. Here a small change of the energy value of the quantum determines a flux of quanta to one fibre system more than to another. At this point there is a maximum rate of change of hue for a given change of wave-length.

(10) An interesting deduction from the present theory is that, under conditions of increasing stimulation with any one colour, there should be change of hue leading ultimately to white sensation. This is so because if the supply of quanta is in excess of what will engage all available fibres there will arise summation of stimuli, and finally the complete activation of all nine fibres. The sensation of being dazzled then arises, and we feel the white sensation, the more normal creation of which is due to simultaneous orderly excitation of two, three and four fibre groups.

(11) The bearing of these views on colour-blindness is as follows:—Following Dr. Parsons, I shall use the term deuteranope to denote the green-blind dichromate, and the term proteranope to denote the red-blind dichromate. To make my remarks more intelligible, I reproduce here (fig. 2) König's and Dieterici's curves.*

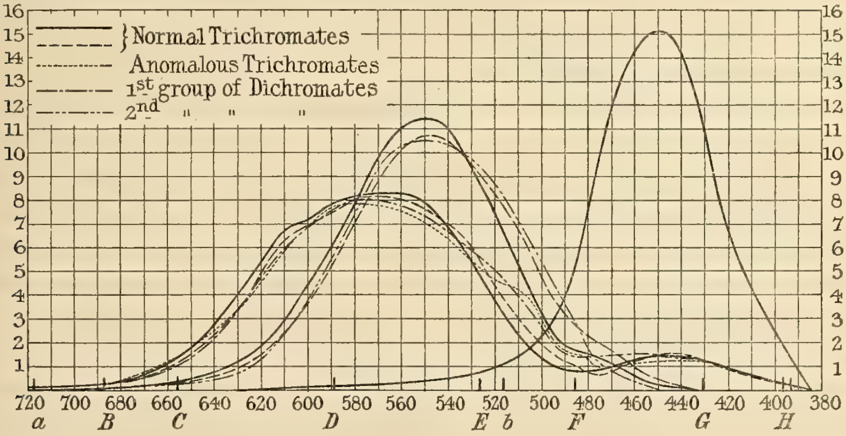

Fig. 2.

The red curve and the blue curve of the deuteranope are normal. We have therefore no reason to suppose his colour interpretive mechanism respecting red and green different from that of the normal retina. He possesses a two-fibre sensation and a four-fibre sensation. He sees his "white" light (grey) at a point on the spectrum, where at the intersection of the two curves both colours stimulate equally. He must possess six fibres leading from his cone, as compared with nine in the case of the normal eye.

It is, I think, easy to understand that this number will not favour the development of a green sensation. White light prevails above all other in Nature, for white is the colour of sun-light. His receptive mechanism, when fully activated by white light, can stimulate but two complete sensations: the blue and the red. Hence, although it is certain that "green" quanta reach his cone, they, as such, find no sensational response in his brain. He is blind to them as stimulants of green sensation. The "green" quanta effect such stimulation of red and blue sensation as takes place in the normal eye; but there is a large residual number of them which are lost to him. The total luminosity of the spectrum is less for him than for normal vision, as is well seen in Abney's curves of photopic luminosity.

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* 'Die Grundempfindungen,' König and Dieterici, Hamburg and Leipzig, 1892.

The red-blind or proteranope possesses a spectral range of colour vision identical with the green and blue of normal colour sight. The maximum height, as given in these curves, is a very little less. The blue curve is normal. Hence we conclude that he possesses three-fibre and four-fibre vision. All that has been said above respecting the eye possessing six fibres applies to the eye possessing a total of seven fibres. The loss of red vision involves a considerable deduction from the total luminosity of the spectrum, as Abney's curves show.

The scotopic or rod-vision of the dichromate is normal. It is the result of a one-fibre stimulus.

The monochromate's case is interesting. Apparently, he possesses but one fibre leading from the cone. His foveal vision is rod-vision. The normal achromatic scotopic curve and the monochromate's curve shown in König's figure are identical. It is to be recalled that this curve is also that of the light absorption of the visual purple and of its rate of bleaching. The monochromate possesses no more than twilight vision.

Cases of so-called blue-blindness are rare, and have generally been pathological in character, as is the case in subjects investigated by König. Little seems known respecting this abnormality. An eye with this defect would possess a total of five fibres, according to the theory under discussion.

(12) In the foregoing pages, and in those yet to come, the unit light stimulus discharged by a single visual fibre is frequently referred to. It represents a very small quantity of energy. Its actual amount is unknown. It must not be confused with the quantum which plays the part merely of the finger on the trigger. This minute quantity of energy discharged into the cerebral cortex evokes our unit of luminous sensation. This is a constant of deep interest to us, and one which dominates our psychology, and, indeed, our very existence as intelligent beings.

I propose to designate it a photon, using the English plural, photons. Symbolically, the letter ϕ will be assigned to it.

(13) The question naturally arises:—Are we to suppose nine fibres quite unco-ordinated in the cone, or are the fibres fused or grouped in bundles so

that we can regard them as functionally reduced to three nerves, the liminal excitatory stimuli of which are in the ratio of 2 : 3 : 4 : units of energy, and which discharge into the visual centre of the brain quantities of energy in the same ratio, that is, 2 : 3 and 4 photons?

The latter assumption certainly facilitates the conception of their manner of functioning. If such is the real state of the case, the cone of the deuteranope possesses but two nerves or nerve-groups, having excitatory stimulus values in the proportion 2 : 4. His green-blindness follows at once, for he does not possess any apparatus competent to discharge an intermediate stimulus—*i.e.*, one of the value of three photons. So also in the case of the proteranope; the missing apparatus is that which discharges two photons in the cerebral cortex, *i.e.*, which creates the red sensation.

It seems, however, possible that nine unco-ordinated fibres offer an equally good explanation of the whole matter. For consider the development of colour-vision in the young child. The three fundamental sensations have never before been experienced, but they are latent, and will in due course arise as part of his consciousness. In the normal eye, white light—which is by far the most abundant and prevalent light in Nature—excites all of the three latent sensations, and in an equal degree. Later, when coloured objects attract the attention of the child, the latent sensations begin to assert themselves separately under the specific stimulus of each colour. Two-fibre stimuli excite the latent red sensation, and so on.

In the case of the green-blind there are only six fibres present. He may possess as an infant all the three sensations latent. However, the ubiquitous white light activates six fibres only. Now these must be two- and four-fibre stimuli, for, admitting that the three quanta possess an equal chance in the first instance of capturing their appropriate number of fibres, these quanta have to compete with two-fibre and four-fibre quanta, and the allocation two and four alone activates *all* the fibres, a state which is characteristic of white light and must arise under photopic conditions. His two-fibre sensation and his four-fibre sensation are, therefore, developed, while a latent three-fibre sensation will become atrophied. Three-fibre (green) quanta will, indeed, as in the normal eye, activate much red sensation and some blue sensation fibre-groups. Otherwise he fails to get any benefit from the quanta. In the case of the red-blind the same causes are operative to retard or annul the education of the red sensation. The anatomical question at issue might possibly yield to histological research.

(14) In order to understand in what manner all the varied hues of the spectrum arise out of the present hypothesis, consider a specific case, say, yellow sensation. Yellow sensation is felt when $2\phi + 3\phi$ are simultaneously

discharged at the visual centre of the brain. That is, we see it at a point on the spectrum where the quanta activate both two-fibre and three-fibre groups in about equal numbers. Somewhat nearer to the red end the quanta, owing to their lesser energy, affect the three-fibre groups in less numbers than they affect the two-fibre groups. So that there are, say, $n \times 2\phi + m \times 3\phi$ (n being greater than m) discharged in the visual centre. This excites an orange sensation. Such an expression as I have just written down is at the basis of colour sensation equations.

The hue at any point, therefore, depends on the destination of the spectral quanta radiated at that point; that is, upon their allocation among the fibre groups which they are competent to activate.

There are a considerable number of distinguishable hues, an appreciable amount of sensation being evoked by a very few quanta.

(15) The stimulus value of the three colour sensations in such proportions as to give white light is nine photons. Two colours are complementary to each other when the sum of their stimulus values is equal to 9ϕ. Thus red $= 2\phi$ is the complement of blue-green $= 4\phi + 3\phi$. Again, green $= 3\phi$ is complementary of $2\phi + 4\phi$, which is a colour not found in the spectrum, *i.e.*, purple. Yellow $= 2\phi + 3\phi$ is complementary of blue $= 4\phi$.

(16) It is a fact that at their achromatic scotopic thresholds all lights are of equal brightness.* This is explicable on the view that rod stimulus is conveyed through a single fibre, and that a one-fibre stimulus is the minimum wherever it originates in the retina. For we are here brought into contact with the "all or none" law. It is not the quantum of energy which is transmitted: the quantum plays the part of the force applied to the trigger. What is transmitted is that unit of energy which the visual nerve generates and discharges into the cerebral cortex. The statement is an assertion of the existence of the photon.

(17) The basis of colour vision may, according to the foregoing views, be stated as follows:—

(*a*) The number of spectral quanta converted to electronic energy and thereby rendered capable of exciting vision is controlled by the light absorption and bleaching of the visual purple (or substance possessing a similar spectral absorption curve).

(*b*) The quanta, increasing in energy from the long to the short wavelengths, stimulate two, three and four fibres of the cone according to their energy, as shown by the colour sensation curves.

(*c*) The simultaneous stimulation of two fibres is attended by the red sensation; of three fibres by the green sensation; of four fibres by the blue sensation.

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* Parsons, *loc. cit.*, p. 61.

(*d*) The unit of luminous stimulus is the nerve discharge of one fibre.

(*e*) No colour sensation is associated with this stimulus.

(18) It seems difficult to question any of these statements if there is any reality in the present theory. But the long-standing question respecting colour sensation confronts us: Can we deal with these cerebral phenomena quantitatively; in a physical sense? It is a question most difficult to answer. Possibly it is ultimately unanswerable. I think, however, the present theory gives a little to go on.

It seems probable that the numbers of photons characteristic of each of the fundamental colour sensations constitute an energy relation between them. The colour sensation curves depict the amount of excitation of three cerebral effects in answer to spectral stimuli. Each sensation is an accompaniment of a particular form of energy stimulus, *i.e.*, of two, of three, or of four photons simultaneously discharged. To the *form* of the stimulus the specific character of the sensation is to be referred. We must be prepared to admit that an energy equivalent to the sensation must exist. *Ex nihilo nihil fit.*

If we measure the maximum heights attained by the three colour sensation curves of König* (or the two colour sensation curves in the case of dichromates) we find them related to the frequencies of the corresponding primary colours; that is the heights are, for the red, green, and blue curves, in the ratios 2 : 3 : 4 approximately. Thus we find for the maximum ordinates (green and blue sensations) of the proteranope the heights 10·8 : 15·2, *i.e.*, a ratio of 3 : 4 approximately, and for the deuteranopes red and blue maxima 8 : 15·2, *i.e.*, a ratio of 2 : 4 approximately. In the trichomate the three colour sensations attain the stimuli values 8·2 : 11·4 : 15·2, which are in the ratio of 2 : 3 : 4 approximately.

This seems to suggest that the quantitative values of the three sensations stand in the ratio of the number of photons originating each sensation. Herein is an energy relation between the colour sensations, if I have viewed the matter rightly.

(19) Luminosity or brightness would seem to be a sensation directly dependent on the number of electrons which stimulate the nerve fibres. This appears to be a necessary inference from the fact that curves of spectral luminosity in general and the curves of absorption and bleaching of the visual purple are in fair agreement.†

We must specially notice the resemblance between the photopic and scotopic luminosity curves.* Now the latter according to the present theory, can represent but one photon from each electron. We must conclude that the former also represents one photon from each electron.

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* *Ante*, fig. 2.

† Parsons, *loc. cit.*, p. 55; compare figs. 1, 10, 11, 14, etc.

(20) From this we must infer, as regards photopic luminosity, that the number of fibres activated by one electron does not influence the luminosity stimulus. The stimulus remains a unit stimulus, irrespective of whether it originates from two, three, or four grouped fibres.

If the stimulus value were proportional to the number of constituent photons, the form of the resultant photopic spectral curve must differ radically from that of the scotopic curve. It would rise towards the violet end. This feature it certainly does not exhibit. Of course, the photopic luminosity curve stands at a higher energy level than the scotopic curve. This is because the numbers of quanta acting per second are greater in the former than in the latter case.

(21) On the other hand, with respect to the excitation of colour sensation, the number of constituent photons entering into the stimulus is all important. For it is by this number, *i.e.*, by the *form* of the stimulus, that the fundamental colour sensations are evoked.

The relation of luminosity to colour sensation is, therefore, according to the present theory, as follows :—Luminosity is the more primitive sensation, and at first was associated entirely with rod vision. The evolution of the cone brought in multiple stimuli, and the sensation evoked became correspondingly complex. The basal luminous sensation remained, excited as before by a nerve stimulus from the retina, but it was accompanied now by a new and additional sensation, that of colour.

Colour sensation necessarily involves luminous sensation. It necessarily involves it because the energy is there which excites it. The converse proposition is not, however, true. It is not true, because there may be sufficient energy to excite a luminous sensation and not sufficient to excite colour sensation. The separation of colour sensation from luminous sensation is therefore impossible. Colour sensation curves take both into account.† Hence the energy relations revealed in the relative heights of the three curves, as referred to above, is to be expected.

It is easy to see that there is a balance of energy available for the colour stimulus. The unit of luminosity is referable to the photon. Red sensation is seen when two photons act simultaneously. We may ascribe one to the luminous and one to the colour sensation. As both arise together, and are, in fact, superimposed, this allocation of the energy is perhaps a little fanciful.

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* Parsons, *loc. cit.*, fig. 14.

† König and Dieterici, *loc. cit.*, p. 22.

However, it must possess a basis of fact, for it affords an explanation of the relative luminosities of the colour sensations. For red, the energies are equally divided; for green, two go to the colour sensation and one to the luminous; for blue, the allocation is three and one. Hence the last is the least luminous of the colour sensations.

(22) Another matter finds explanation in these considerations. The colourless interval between the general threshold and the colour threshold is for long wave-lengths (beyond 670 $\mu\mu$) almost completely absent. "In fact, even with very good dark adaptation, such a red light excites the sensation of red."* The small energy values requisite to excite both colour and luminous sensations in the case of red sensation explains this observation. Very feeble electrons served to excite both. With less luminous colours it is otherwise. In these cases, a relatively small number of the electrons liberated from the sensitiser carry into the cone the requisite energy to build up the stimulus to the value of the colour sensation. For the blue sensation, the colourless interval is therefore the greatest.

(23) On these views white is a sensation arising in the loss of form attending the activation of all nine fibres. This loss of form causes it to resemble the luminous sensation. It is neither a true luminous nor yet a true colour sensation, but something sharing the properties of both. It is a nine-fibre sensation, *sui generis.*

(24) One of the most remarkable facts of vision is its marvellous range: from the feeblest twilight to full mid-day sunshine. In photopic vision a very large number of electronic stimuli probably do not and cannot take effect, even if we assume a very brief refractory period. The figures are instructive.

I assume at least nine nerve fibres, discharging stimuli, in each cone, and that the normal refractory period is 10^{-4} sec. A total of 9×10^4 stimuli can be accepted per second per cone. If we estimate the number of cones in the fovea as 2×10^4, this area might transmit 18×10^8 stimuli. The number of electrons or quanta involved is one-third of this where white light is concerned; or, say, 6×10^8 quanta per second.

A standard candle gives $5 = 10^5$ ergs per second (Rayleigh), and from this 4 ergs per second reach 1 sq. cm. at a distance of 1 m. Assume a ten-candle-power light, and that the pupil has closed down to the area of 3 sq. mm. Further, suppose the image of the source of light just covers the fovea. Under these conditions 0·22 ergs reach the fovea in one second.

Now the value of a "green" quantum is about 5×10^{-12} erg. Hence the energy reaching the fovea will give rise to about 4×10^{10} quanta or electrons per second, or about seventy times the possible transmission. Of course there would be some loss by absorption in the media of the eye, and, again we may not limit the refractory interval as we have done. It might be ten times smaller and yet not fall below the value apparently associated with audition. But even so, we must recognise that in photopic vision the flash of the electron may often come at the wrong moment and fail to evoke vision.

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* Parsons, *loc. cit.*, p. 61.

An important deduction from this consideration is that a highly dilute visual purple may suffice for the requirements of photopic vision. The fact that dark adaptation of the fovea is feeble supports this view.

The Effect of Red Fatigue on the White Equation.

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The white equation, that is the amount of pure spectral red, green, and violet, required to match a simple white, is of fundamental theoretical importance. The apparatus used in these experiments is of similar principle to that of Abney, namely, the selection of portions of the spectrum by slits and their re-combination on a white surface by means of a lens, but with several improvements suggested by Captain Fulton. The source of light is a "Pointolite" lamp of 1000 c.p. The light is focussed on the slit of a collimator, from which it emerges in a parallel beam. It is then dispersed by a compound prism of the Amici type. A lens placed close to the prism focusses the spectrum on the slits, the light being reflected by a mirror placed in the path. A second lens is constructed so as to take the whole spectrum, portions of which are isolated by means of slits. The focal length of the lens is arranged so that an image of the last surface of the prism is projected on a screen, the colour being dependent on the portion or portions of the spectrum isolated by the slits. In order to obtain a long light-path, the light is again reflected by a second mirror before reaching the screen. The intensity of a comparison patch of white light is regulated by an adjustable diaphragm placed in the path of light. The apparatus is used in a dark room free from stray light.

The three lights used in these experiments were a red of λ 6670–λ 6770 Å., a green of λ 5144–λ 5156 Å., and a violet of λ 4250–λ 4267 Å. In making the equation, the red and violet slits are kept unaltered, the equation being made by closing or opening the slit, allowing green light to pass. The size of the portion of spectrum in the green for the normal equation is given above, and corresponds to thirteen scale divisions. The equation is very easy to make, the mean deviation being very small.

The following are the results of twelve consecutive observations by two normal-sighted persons, the equations being made alternately, starting with too much or too little green in the equation:—

A. From green...	From red...	B. From green...	From red...
13	13	13	12·5
13	13	12·5	13
13	13·5	12·5	13

One scale division at λ 515 corresponds to about 1 Å.

The screens, which are viewed at a distance of 4 feet, so that the images shall fall on the foveæ, are coated with magnesium oxide. The experiments were made in order to find out the effect of fatigue with red light, which is supposed to affect only the hypothetical red sensation.

The light used for fatigue was a "Pointolite" arc of 100 c.p. with a condenser, viewed through a deep red glass, which only allows red from λ 630 μμ to the end of the red end of the spectrum to pass. The light was viewed with both eyes, and care was taken that the arc should not be directly visible. An ordinary incandescent electric lamp obscured by ground glass was also used. Red isolated in my spectrometer of the region λ 670 was also used with the same result. After a certain amount of fatigue, the length of time varying with different persons, the mixed and the simple lights were viewed. There was now a striking change in the mixed white, which appeared a brilliant green, and no longer matched the simple white. In order to match the simple white, the green had to be so reduced that the mixed light appeared red to a person with an unfatigued eye. Several normal-sighted persons were then tried with the same result. Observations were taken with the equation correct for the observer, and with too much or too little green in the equation. My normal equation is: 36 red, 14 green, and 42 violet; after 5 seconds' red fatigue, the equation was: red 36, green 7, violet 42.

The following observations are by two normal-sighted persons. A, normal equation: red 36, green 13, violet 42; after 30 seconds' red fatigue, the equation was: red 36, green 8, violet 42, and the equation made was exactly the same, whether the normal equation was viewed or an equation in which there was too much or too little green. The observer did not know which was shown. B, normal equation: red 36, green 12, violet 42; after 20 seconds' red fatigue, the equation was: red 36, green 5, violet 42, when his normal equation was viewed; red 36, green 5½, violet 42, when the equation viewed at first contained too much green; and red 36, green 4, violet 42, when the equation viewed at first contained too little green.

It will be seen that, after fatigue with red, only about half as much green is required in the equation. In making these observations, it is important that the fatigue should not be too strong, as the blue-green after-effect may obscure the appearance, and prevent correct observations being taken. A man with normal colour vision, who had passed my card test, had a normal white equation, normal complementary of yellow and violet, and normal monochromatic division of yellow, after one minute's red fatigue, was quite satisfied when the green slit was quite closed or open to its full extent, the equation then appearing bright red and green respectively to a normal-sighted person. This is in accordance with the observations of A. W. Porter and myself on successive contrast,* when we found that the after-image was not affected by subsequent light falling on the retina when this was not of too great intensity. When the fatigue is great, a blue-green after-effect is seen, which is negative in colour but positive in luminosity, appearing bright on the dark ground; it appears to consist of minute particles, which move towards the centre with a whirlpool movement and then disappear. If the equation be then made, it will be found, as before, that the proportion of red is too high. A colour-blind man who put too much green in the white equation, and another who put too little, after fatigue with red put a much less amount, thus varying in the same way as the normal.

It is generally stated that, with regard to the fovea, it is found that all colour matches still remain valid, no matter what kind of light may have previously stimulated the retina. This appears to be true when red of the region λ 780 is used for the fatigue. This was obtained by viewing the light through a deep violet glass combined with a deep red glass. The two glasses only allow red of the region of λ 780 to pass. Watson† stated that colour matches remain valid after stimulation with another light, but that the mean deviation was considerably increased. This does not appear to be the case when the fatigue is not excessive. The blue-green after-effect appears to be due to the decomposition products of a photo-chemical substance (the visual purple) in the retina, and to be distinct from fatigue of the retino-cerebral apparatus. There seems to be considerable variation in the length of time necessary to cause fatigue with different persons.

These observations are quite inconsistent with the three-sensation theory. The white equation and its match cannot be due to similar physiological processes, or both would change in the same ratio.

I must express my indebtedness to Captain Fulton and Mr. Isaacs, of the Board of Trade, for their help in making these observations.

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* 'Roy. Soc. Proc.,' B, vol. 85, p. 434 (1912).

† 'Roy. Soc. Proc.,' A, vol. 89, p. 235 (1913).

*Dictyokinesis in Germ Cells, or the Distribution of the Golgi Apparatus during Cell Division.**

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[Plates 17 and 18.]

Introduction.

In the vast majority of animal cells so far properly studied, two categories of cytoplasmic inclusions have been identified, namely, the mitochondria and the Golgi apparatus.

The Golgi apparatus generally takes the form of an excentric juxta-nuclear system or network, composed of rodlets, platelets or beads, arranged, in many cases, around and over the surface of the centrosphere or archoplasm, in which lies embedded the centrosome. In highly differentiated cells such as the oocyte or nerve ganglion cell, the Golgi apparatus becomes dispersed into the farthermost parts of the cell-cytoplasm, and in most cases therefore loses its relationship to the centrosome.

The equipment of an ordinary cell so far as cell organellæ are concerned, may be stated to be:—(*a*) chromosomes, (*b*) nucleoli, (*c*) Golgi apparatus, (*d*) mitochondria, (*e*) centrosome and centrosphere. During cell division or cytokinesis, all these bodies or organellæ are distributed between the daughter cells in some special manner. In this paper we have investigated carefully for the first time the fate during mitosis of the internal apparatus of Golgi, in a number of animals, from both Vertebrate and Invertebrate Phyla.

Material and Methods.

The material used had been prepared by one of three methods: a formalin-silver nitrate, Golgi apparatus technique; a Kopsch method, or chrome osmium and iron alum hæmatoxylin. The Cavia material was prepared by the Mann-Kopsch-Altmann technique (Gatenby), the Mus testis by Da Fano's cobalt nitrate formalin and silver nitrate modification of Cajal's method, the Stenobothrus testis by the latter technique (Cajal), and the Mollusc gonads by Kopsch, and by the chrome osmium methods. It will be clear therefore

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* Part of the materials used for this research were purchased by a Government Grant of the Royal Society, for which we express thanks.

that we have based our results on a broad foundation so far as concerns the number of forms used, and the techniques by which the cells were prepared.

The sections were prepared by J. B. G. and studied by both of us, but R. J. L. is responsible for nearly all the drawings, and for a great share of the task of finding mitoting cells. The drawings were made with the camera lucida, and after careful discussion and an examination of numerous examples, and great care has been taken not to vitiate our results by careless observation. This has been all the more necessary because the research has offered considerable difficulties.

We have to thank Dr. Da Fano for handing over to us his material of Mus testis from which the case of the rat spermatocyte divisions have been described.

Note on the General Appearance of Resting and Mitoting Cells.

Everyone who has worked with silver nitrate and osmic acid techniques for the demonstration of the Golgi apparatus is well aware that these methods may occasionally prove capricious; but that such techniques really do demonstrate bodies which are not artefacts and which can be seen *intra vitam*, can easily be shown by comparing the images got by such methods, with those live cells of the gonad of Helix or other molluscs. This should still for ever the murmurings of the slipshod critics who have urged that silver nitrate techniques should at all times be avoided.

It is nevertheless true that quite often the formol-silver nitrate, and the Kopsch techniques fail to demonstrate the apparatus, and this applies especially to the Golgi apparatus of mitoting cells. This is the first point which we wish to emphasize: it is possible to find resting and mitoting cells side by side, with the Golgi apparatus beautifully clear in the former, and apparently altogether absent in the dividing cells; that the Golgi apparatus is *not* really absent from the latter can be shown by the examination of better impregnated material in which an apparatus will be found in mitoting cells. But it seems quite certain that during mitosis, in some cases even in the early prophases, the Golgi elements are altered in some way which makes their demonstration quite difficult.

The Golgi Apparatus in the Interkinesis Stage.

As we have already noted, our material consisted of *Stenobothrus viridulus* (a cricket) testis, of Helix and Limnæa ovo-testis prepared by Kopsch and Cajal methods, of Rat and Cavia testis prepared by an osmic and a formalin silver method. We possess also preparations of animals of every other class in the Animal Kingdom (excepting Protozoa) which show the

Golgi apparatus. In nearly all the cases with which we are familiar, the Golgi apparatus, when demonstrated either by an osmic or chrome osmium iron hæmatoxylin method, is found to consist of distinct rods or batonettes—the dictyosomes of Perrincito*—distributed over the surface of the centrosphere or archoplasm. (See Plate 18, fig. 14.)

In other cases the Golgi elements appear to be of a semi-liquid consistency, easily altered by anything but the most delicate treatment in both fixing and embedding materials. In many examples of mammalian cells examined by us, the Golgi apparatus seemed to be merely a cortical stainable area of the archoplasm or centrosphere, but whether this is really the case, *intra vitam*, is very difficult to decide. In other cases (Plate 18, fig. 14), separate dictyosomes or distinct cortical areas were demonstrable.

Dictyokinesis in Mus.

The division of the Golgi apparatus in the male germ cells of *Mus rattus* was worked out in preparations made by the silver impregnation method of Da Fano. The apparatus in the spermatogonia and primary spermatocytes appears as a compact mass of short black rod-like granules, embedded on a darkly-stained archoplasmic mass or centrosphere, as is shown in fig. 1, Plate 17. During the growth phase of the spermatocyte, the apparatus increases somewhat in size, and about the time when the membrane of the nucleus has broken down, preparatory to the first reduction division, the archoplasm can be seen in process of fission. At GA (in fig. 2) the archoplasm is seen separating into two portions. It will be noticed that as the two halves of this substance separate, they carry with them about an equal number of the Golgi rods or dictyosomes. In fig. 3 the archoplasm is completely separated into two constituent parts.

There is no doubt that the cause of this separation is the division of the centrosome, for the two masses move apart and come to occupy positions at opposite ends of the cell, as is shown at GA in fig. 7, which represents a late prophase of a second spermatocyte division. Between the two separated masses of archoplasm arise the fibres of the spindle, and, as the chromosomes become arranged equatorially upon this, the Golgi rods break away from the archoplasm and become scattered in the cytoplasm. Fig. 4 shows this scattering processs during the metaphase. It seems almost as though some force which the archoplasmic mass or centrosome has previously exerted upon the dictyosomes is diverted during the meta- and telo-phase stages to act upon the chromosomes and draw them apart (R. J. L.). Whether or not this be the case, it can be seen that during the meta-, ana-, and early telo-phases, the dictyosomes are scattered irregularly in the cytoplasm, and during the late telophase, when the chromosomes are completely separated off into the two daughter cells, the dictyosomes come together again and become attached once more to the archoplasm. Early and late telophases are shown in figs. 5 and 6, and the reconstructed compact form of the apparatus is shown in the case of the recently formed spermatid in fig. 8.

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* 'Arch. Ital. Biol.,' vol. 54 (1910).

Both in the primary and secondary spermatocyte divisions, the process of dictyokinesis is essentially the same. About the time that the nuclear membrane breaks down, the archoplasm, with the dictyosomes, divides. During the metaphase the dictyosomes become scattered, and towards the end of the telophase they are attracted once more to the archoplasm, to the surface of which they become attached.

The *rôle* of the Golgi apparatus from the spermatid stage onwards has already been described by Gatenby and Woodger* in the case of Cavia. The greater part of the mammalian Golgi apparatus has been shown to be sloughed off during the formation of the spermatozoon, but a few dictyosomes remain attached to the middle piece. The beginning of the sloughing off process in the Mus is shown in fig. 9.

Dictyokinesis in Cavia.

Dictyokinesis in *Cavia cobaya* is, with few exceptions, essentially the same as in the case in Mus. The process in this mammal has been studied in preparations made by Weigl's modification of the osmium tetroxide method of Kopsch.

During the early phases in the development of the spermatocyte, the archoplasm to which the dictyosomes are attached shows a remarkable frothing. This is shown at "*b*" in fig. 16, and again at VV in fig. 12. Small "bubbles" appear to arise peripherally upon the archoplasm; they increase in size and, owing to the fact that later they disappear, it would seem that they burst and discharge their contents into the cytoplasm. Peripherally, these bubbles are surrounded by a substance which stains black with osmium tetroxide. At the end of the diakinetic stage of the chromosomes the formation of these "bubbles" upon the archoplasm ceases, and the dictyosomes are then seen as semilunar rods attached peripherally to the archoplasm, as is shown in fig. 16 at "*a*."

Before the nuclear membrane disappears the archoplasmic mass, which has enlarged during the growth period, begins to divide in exactly the same manner as in Mus. The beginning of division is shown in figs. 13 and 14.

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* 'Quart. Jour. Micr. Science,' 1921.

The divided apparatus is shown in fig. 15. In fig. 17 is seen a metaphase stage, the Golgi elements scattered in the cytoplasm appear as annular bodies composed of two semi-lunar rods. Mitochondria have been drawn in this figure as they appear in preparations counter-stained with Altmann's acid fuchsin. During the telophase, when the chromosomes are completely drawn apart, the dictyosomes are attracted once more to the astrosphere. Their movement towards the astrosphere at this stage is shown in fig. 18.

The second spermatocyte division takes place in exactly the same manner as the first. The divisions of the archoplasm, and its movement to the opposite ends of the cell during the prophase, appears to take place with considerable rapidity, as very few cells are found in these stages, and it will be seen from the figures that but little change occurs in the chromosomes during fission and separation of the archoplasm.

Division of the Chromatoid Body in the Mammals Mus and Cavia.

At the same time as the Golgi apparatus of the mammalian spermatocyte is in process of division, the chromatoid body breaks up into two equal parts. This process is best seen in the Weigl-Kopsch preparations of Cavia, in which, after staining with acid fuchsin, the chromatoid body appears coloured similarly to the mitochondria. In fig. 13, which shows the beginning of dictyokinesis in the primary spermatocyte of Cavia, a single large chromatoid body (CB) is seen. In fig. 14, where the archoplasm appears very nearly divided into two parts, the chromatoid body has divided, and the two parts are just separating. They are further apart in fig. 15, while, in the metaphase stage shown in fig. 17, they occupy positions on opposite sides of the chromosome group. A similar stage in the spermatocyte of Mus is shown in fig. 4. At the telophase, the two chromatoid bodies become separated off into the newly forming cell, as is happening in fig. 18. Consequent upon the repetition of this process in the second spermatocyte division, each spermatid has a single chromatoid body directly derived from that of the spermatogonium.

Dictyokinesis in Molluscs.

The fundamental nature of the dictyokinesis in the germ cells is shown by the similarity of the process in animals so widely apart in their systemic position as the Mollusca and Mammalia.

At fig. 19 is drawn a primary spermatocyte of Helix as it appears in Flemming-without-acetic preparations, stained by iron alum hæmatoxylin. The chromosomes have reached their diakinetic stage, and mitochondria, which previously were rounded, have metamorphosed to form rodlets.

The archoplasm is seen just dividing, and carrying with it the semi-lunar Golgi rods. The two parts of the apparatus are carried to opposite ends of the cell by the centrosomes, and the spindle arises between them in the normal manner.

An early anaphase in the primary spermatocyte of the mollusc Limnæa is shown in fig. 20, which is drawn from a Kopsch preparation. It will be noticed that, while the chromosomes are being drawn apart, the dictyosomes are breaking away from the asters. Later they become more scattered, and then, when the chromosomes have reached the poles of the spindle, the dictyosomes are drawn once again to the archoplasm.

The same process is repeated during the second spermatocyte division, so that, in the spermatid, the Golgi apparatus is in the clumped condition. In the formation of the spermatozoon, it is sloughed off along the tail, and is seemingly absent altogether in the mature spermatozoon.

Dictyokinesis in Stenobothrus.

Dictyokinesis, as observed in the spermatocyte of the cricket, *Stenobothrus viridulus*, differs from that described for the previous animals, in that the Golgi elements break away from the archoplasm at a very early stage in the development of the spermatocyte. In Cajal preparations, the apparatus in the spermatocyte appears first as a number of black granules around the archoplasm. Soon these granules break away and scatter in the cytoplasm, leaving the archoplasm, as is shown in fig. 10.

The end of a typical first spermatocyte division is shown at fig. 11. It will be seen that the Golgi elements are fairly evenly distributed in the secondary spermatocyte. After the second spermatocyte division, many of the dictyosomes become once more attached to the archoplasm, and they remain thus in the spermatid.

Discussion.

It seems to be agreed by most observers that, during mitosis, the chromosomes divide longitudinally, that is, if they are elongated in shape. Bolles Lee,* in a recent paper, has dropped a bombshell into the camp of the chromosome theorists and Mendelians, by suggesting that "there is no longitudinal splitting. The division is a transverse one, brought about by a folding of the chromosomes at their middle, and their ultimate segmentation at the bend there formed." It is not our intention at the present juncture to join in this discussion, but, for the purposes of this paper, it may be said that, whether the division of the chromosomes be transverse or longitudinal, it is at least a very equal one quantitatively.

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* 'Quart. Jour. Micr. Science,' 1921.

In this present paper, we have shown that, in animal germ cells, the Golgi apparatus may during interkinesis be spread throughout the cell cytoplasm, as in Stenobothrus, or it may be excentric and juxta-nuclear, as with most of the forms herein described. In a previous communication, one of us showed that, in the case of *Limax agrestis*, the number of dictyosomes or Golgi (nebenkern) rods in the spermatocyte is almost always eight, and, after the two maturation divisions, each of the four spermatids has two rods.

The size of the rod in the spermatid is the same as that in the spermatocyte, and this, with the evidence brought forward in this paper, shows that each rod passes through the maturation divisions undivided. It had previously been shown that the number and size of the dictyosomes or Golgi rods in such a mollusc as *Helix aspersa* may vary greatly.*

Multiplication of the number of the Golgi rods may take place during the interkinesis stages, as was demonstrated clearly in the case of the Limnæa egg,† but no actual division of individual rods seems to occur at cytokinesis, but only a sorting out of whole rods. This is well shown in Plate 18, figs. 19 and 20 of this paper, and was first worked out by J. A. Murray.‡

When we come to the case of some of the mammals, it will be noted that, even though the molluscan dictyokinesis may be haphazard and unequal, that of the mammal is even more irregular. That there is a true dictyokinesis or distribution of Golgi elements during mammalian cytokinesis cannot be doubted, but there are several relative points which should be brought to notice. In the first place, the Golgi apparatus of such forms as Mus and Cavia often appears to be a mere cortical layer plastered upon the surface of the centrosphere, separate dictyosomes or rods of any kind being apparently absent. In other cases there are distinct dictyosomes, but these are rarely so well marked as in molluscan germ cells. Dictyokinesis in the mammalian germ cells studied by us is never so much a sorting out of distinct rods as in molluscs, as an irregular breaking up of possibly only that part of the archoplasm or centrosphere in close proximity to the more cortically disposed Golgi substance. It is only right to point out, however, that we have found some very clear metaphases where the Golgi elements were composed of discrete dictyosomes, as in Plate 77, fig. 3.

But we come to another point; perusal of such work as that of Lenhossék§ on the rat will show that such observers figure maturation metaphases with a single free unbroken centrosphere at one pole of the spindle; in other words, according to Lenhossék and such workers, the centrosphere does *not* divide as we have drawn in Plate 17, figs. 1–7, of the present paper. But in our own preparations of the rat, we never definitely found such an undivided centrosphere at metaphase, and can only conclude that Lenhossék is wrong. Our figures 1–7 of the rat are carefully drawn and are typical of many examples we have studied.

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* See 'Quart. Jour. Micr. Science,' vol. 62 (1917), Plate 32, fig. 25, and Plate 33, fig. 32.

† 'Quart. Jour. Micr. Science,' 1919.

‡ 'Zool. Jahrb.,' vol. 11.

§ Quoted and figured by Doncaster in his 'Cytology.'

In molluscs the Golgi rod or dictyosome is evidently a compact and definitely shaped body possibly formed of some proteid substratum, but in the case of the mammalian germ cell the Golgi element appears to be more fluent, and less able to keep its shape during dictyokinesis. That this is so, seems all the more likely from evidence procured by examining the results of different fixing techniques on the apparatus. The Golgi apparatus dictyosome of the mammalian germ cell during the metaphase always appears to show a tendency to become spherical or ovoid, which indicates that it must be nearly of the same viscosity as the ground cytoplasm in which it lies, assuming of course that the protoplasm is still a sol at this period.

Chambers' work on the dividing sea-urchin's egg may not apply to maturing germ cells, but we do not know at present. At all events it seems indicated that the Golgi substance of the mammalian germ cell is of a more liquid nature than that of the mollusc.

There is then another matter to which we wish to turn: although many of the older workers figured Golgi rods or dictyosomes in the resting cell, they rarely found any apparatus during kinesis. Take for instance the work of Tullio Terni.*

It is quite certain that some change comes over the Golgi elements during cell division; in molluscs, insects and mammals, in certain worms such as Saccocirrus, and in all the examples we have observed, it has been found that it is possible to find mitoting and resting cells side by side, in *either* of which the apparatus may apparently be absent—but only apparently.

The case of the mollusc spermatocyte is classical; short mordanting in chrome osmium, and curtailed staining in iron hæmatoxylin is always sufficient to show the Golgi rods of resting cells, but this treatment will often prove insufficient to bring into evidence the apparatus of mitoting cells. But prolong the mordanting, and the staining, and the story becomes quite different—this is what observers like Terni have failed to do.

To explain this behaviour of the Golgi element is difficult, yet in it we see possibly the one solution. In the case of the chrome osmium technique it seems that the difference is due to a withdrawal from the Golgi elements at the prophase and metaphase, of some lipoid substance to which the heavier and more facile staining of the resting stage is due. In the case of the Weigert stain of medullated nerve fibres, the hæmatoxylin forms a lake with oxidized $(K_2Cr_2O_7 + OsO_4)$ lipoids in the sheath. We think that such may explain the behaviour of the Golgi substance at cytokinesis. The lipoids thus withdrawn from the Golgi elements might serve some purpose during cell division. In this connection one remembers Boveri's view that the archoplasm or centrosphere supplied the material for the formation of the mitotic spindle.

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* 'Arch. f. Zellf.,' vol. 12 (1914).

The Significance of Dictyokinesis.

It has been shown here that the process of dictyokinesis is generally extremely haphazard, and in contrast with karyokinesis does not entail any sort of fission of individual elements as occurs with the chromosomes, but merely an unprecise sorting out of parts of the Golgi reticulum, between the two daughter cells.

It seems indicated that the Golgi apparatus is an integral and necessary part of the cell constitution, but during cytokinesis or cell-division, there is to be found in connection with the Golgi apparatus nothing comparable with the exact division of the chromosomes. The distribution of the Golgi apparatus between the daughter cells, as we have shown, takes place by a more or less rough process of sorting out of individual pieces or spheres formed by disintegration of the apparatus during the prophase of mitosis, and this process entails no division of the individual elements or dictyosomes, as occurs in the chromosomes of a somatic mitosis.

All this seems to indicate that the Golgi apparatus takes a part less precise and less important, in the hereditary processes of the cell, than that fulfilled by the chromosomes.

DESCRIPTION OF PLATES.

Explanation of Lettering.

AR ...	Archoplasm.
CB ...	Chromatoid body.
CH ...	Chromosomes.
GA ...	Golgi apparatus.
M ...	Mitochondria.
VV ...	Vacuoles associated with the Golgi apparatus and Archoplasm.

PLATE 1.

Figs. 1 to 9 were drawn from preparations of the testis of the rat which had been fixed by Da Fano's method and counterstained with carmine.

Fig. 1.—Spermatogonium, showing the Golgi apparatus at GA in the compact form.

Fig. 2.—Late prophase of the primary spermatocyte division. The nuclear membrane has broken down, and the Golgi apparatus (GA) is dividing.

Fig. 3.—Primary spermatocyte division, showing the two parts of the Golgi apparatus (GA) moving apart.

Fig. 4.—Metaphase in primary spermatocyte division. The chromosomes (CH) are arranged equatorially and the Golgi rods are scattered in the cytoplasm. The chromatoid body is shown at CB.

Fig. 5.—Early telophase of primary spermatocyte. The Golgi elements are scattered irregularly.

Fig. 6.—Late telophase of second spermatocyte division, showing the two separated groups of chromosomes (CH), and the dispersed dictyosomes (GA).

Fig. 7.—Late prophase of secondary spermatocyte division, showing the two archoplasmic masses with adherent Golgi apparatus (GA) at opposite poles of the cell.

Fig. 8.—Spermatid with reconstructed compact form of the Golgi apparatus (GA).

Fig. 9.—Spermatozoon with the Golgi apparatus before becoming sloughed off.

Figs. 10 and 11 were drawn from Cajal preparations of the testis of *Stenobothrus viridulus* stained with fuchsin.

Fig. 10.—Spermatocyte, showing the archoplasm before division, and the numerous scattered Golgi elements (GA).

Fig. 11.—Late telophase of first spermatocyte division, showing the elements of the Golgi apparatus (GA) fairly equally distributed in the secondary spermatocytes.

PLATE 2.

Figs. 12 to 18 were drawn from Weigl-Kopsch-Altmann preparations of the testis of Cavia. Mitochondria are shown only in fig. 17, but are present at all stages.

Fig. 12.—Spermatocyte showing the curious vacuoles (VV) associated with the Golgi apparatus.

Fig. 13.—Primary spermatocyte at late prophase with the Golgi apparatus beginning to divide and a single chromatoid body (CB).

Fig. 14.—Later stage of the prophase, the Golgi apparatus is in process of fission, and the chromatoid body (CB) has just divided.

Fig. 15.—Primary spermatocyte with the apparatus (GA) divided into two parts. The nuclear membrane has just broken down, and there are two chromatoid bodies present.

Fig. 16.—Two forms of the Golgi apparatus, as found in the spermatocytes of Cavia. In (*a*) it consists of a number of semi-lunar dictyosomes attached peripherally to the archoplasm. In (*b*) it is much more darkly impregnated with the osmium tetroxide and a number of frothings appear on its surface.

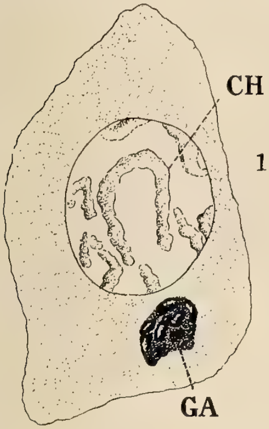
Fig. 17.—Metaphase of first spermatocyte division. The Golgi elements (GA) are scattered unevenly in the cytoplasm, the chromatoid bodies are seen at CB, and granular mitochondria (M) are fairly evenly distributed throughout the cytoplasm.

Fig. 18.—Telophase of second spermatocyte division, showing the Golgi elements (GA) moving towards the archoplasm, and the chromatoid bodies at CB.

Figs. 19 and 20 show the spermatocytes of Molluscs. The former is from a Flemming-without-acetic preparation, stained by iron alum hæmatoxylin: and the latter from a preparation by the Kopsch method.

Fig. 19.—Primary spermatocyte of *Helix aspersa* at the diakinetic stage. The Golgi apparatas has just divided, and the two archoplasmic spheres are beginning to separate. Filamentous mitochondria (M) are seen scattered throughout the cytoplasm.

Fig. 20.—Metaphase of the first spermatocyte division of *Limnæa stagnalis*, showing the Golgi elements (GA) attached peripherally upon the asters at both poles of the nuclear spindle.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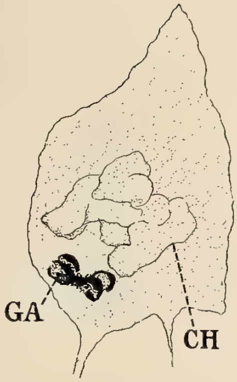
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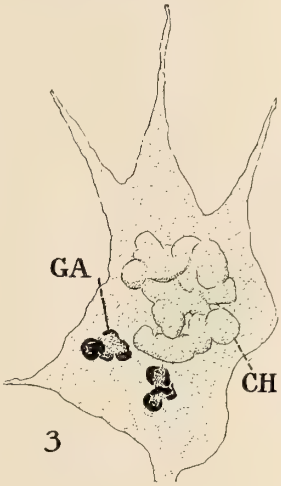
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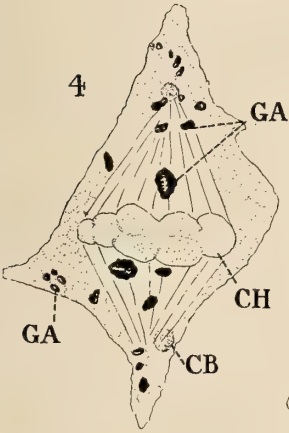
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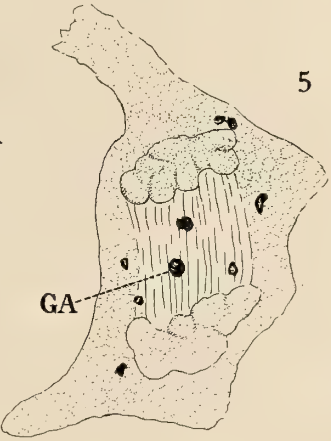
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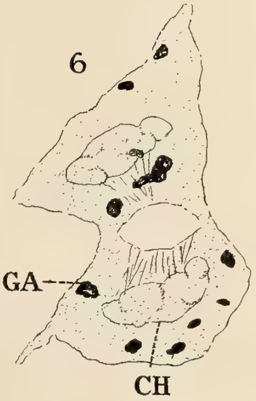
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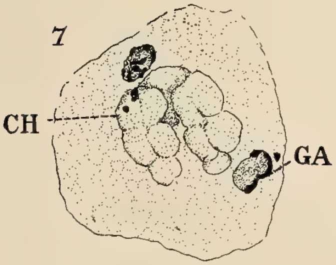
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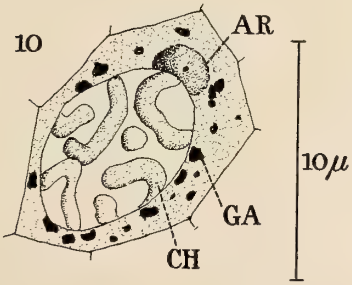
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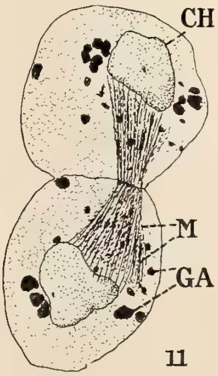
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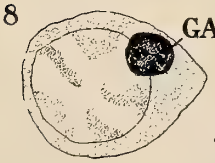
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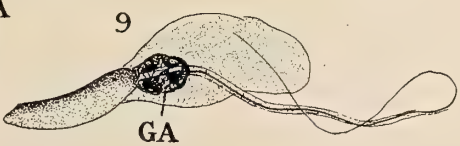
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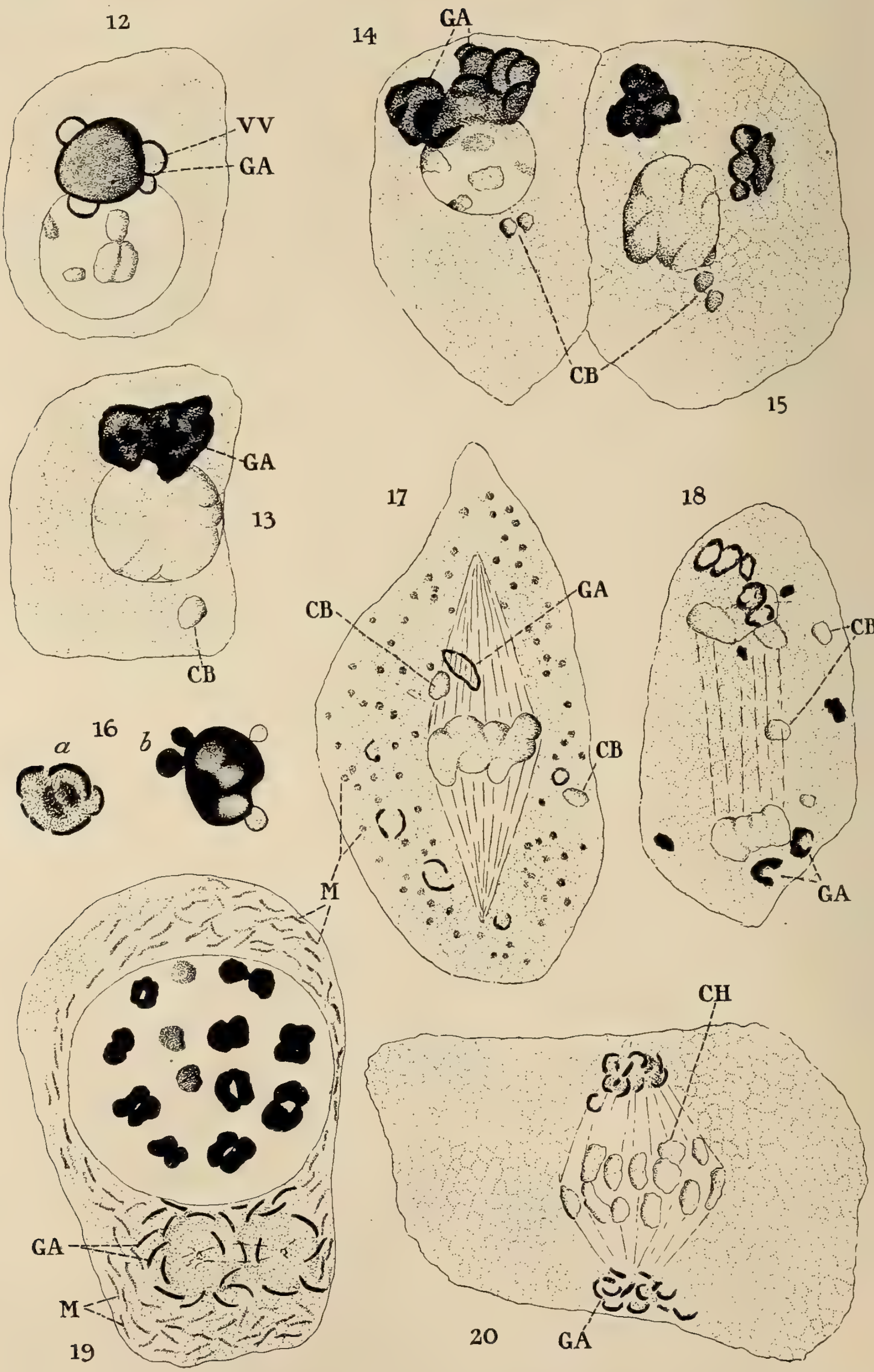
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Break-shock Reflexes and "Supramaximal" Contraction-response of Mammalian Nerve-muscle to Single Shock Stimuli.

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The following observations continue some of those reported previously to the Society by Mr. N. B. Dreyer and myself (1) and by Dr. K. Sassa and myself (2). But in the present instance the technique has been considerably modified in the hope of securing finer discrimination between the contraction forms obtained. The speed and intensity of the reactions of mammalian muscle with its blood supply intact rendered desirable greater lightness in the moving parts of the myograph. The free vibration rate of the isometric myograph now used has been more than 900 a second; its damping such that when suddenly released from a torsional deviation, approximately that obtaining in the muscle observations, the vibrations ceased to be visible under tenfold enlargement in about 0·03 sec. The registration of the myograph movement has been by optical projection on a travelling photographic plate, time being recorded on the plate by a rotary shadow-marker of the pattern devised by Mr. Bull, of the Institut Marey, Paris.

The reflex preparation has been *tibialis anticus* muscle in the spinal cat, decerebrated and free from drugs. The stimulus used has been a single break-shock given by an automatic key and applied by platinum electrodes 5 mm. apart to the central stump of the cut and isolated afferent nerve (popliteal, internal saphenous or digital branch of musculocutaneous) or, for the motor control, to the peripheral stump of the cut motor nerve (peroneal), kathode proximally for the former, kathode distally for the latter. The magnification of the muscle movement has been sixty times: that is, for a myogram of 30 mm. height recording a tension of 915 grm., the muscle, *e.g.*, of 12 cm. length, shortened 1/240 of its length; the myograms are thus practically isometric.

The trend of the results obtained can be conveniently indicated by describing briefly the changes observable (Fig. 1) in the reflex myogram under progressive step-by-step increments of strength of the break-shock delivered as stimulus to the afferent nerve, *e.g.*, popliteal. Starting with a break-shock of little above threshold value for reflex stimulation, *e.g.*, the secondary coil at 60 cm. from primary on the inductorium scale, the reflex myogram then obtained is of lower crest-height and less steep ascent than is the maximal-twitch myogram (subsequently obtained by break-shock stimulation of

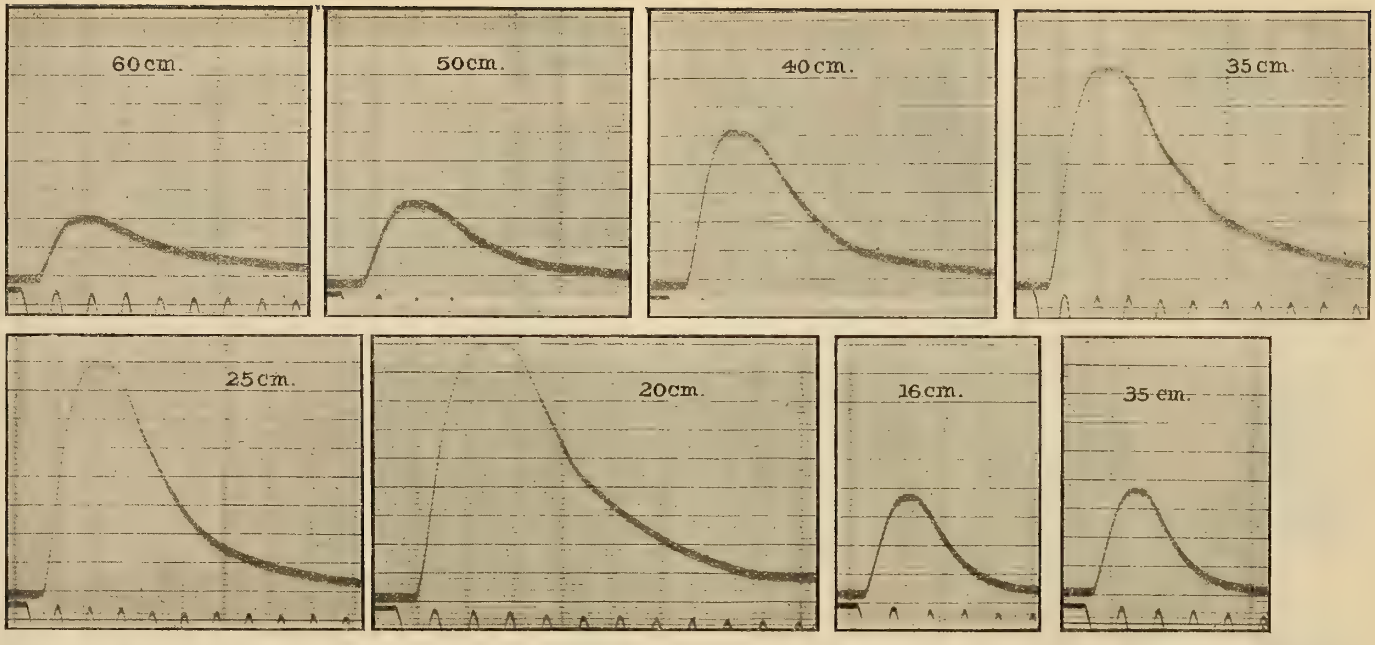

Fig. 1.—Reflex contractions of tibialis anticus (spinal cat), each excited by a single break-shock to central stump of cut popliteal nerve, the coil distance in centimetres on inductorium scale shown by numerals against the corresponding inset in the figure. Lower two insets to right are maximal twitches to single break-shock to cut motor nerve, coil distances similarly indicated. Time ordinates $\frac{1}{25}$ sec. apart each $\frac{1}{5}$ sec. shown by stronger vertical. The horizontals, 1 mm. apart, indicate tensions of the isometric record; value of each 1 mm. interval is approximately 31 grm.* Magnification of muscle-shortening is 60 times. Stimulus-signal below.

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* Throughout the figures, except fig. 3, the "shadow myograph" was arranged to give a record 50 mm. in height for a tension of 1500 grm. The tendency of the torsional excursions to become somewhat less relatively with successive equal increments of torsional pull was counteracted by setting the rectilinear shadow's fall upon the camera slit in such a way as to slightly increase the optical magnifications with progressive deviations of the shadow from its zero position. The calibration of the records after this had been done gave the following results: 5 mm. vertical height = 150 grm. tension; 10 mm. v.h. = 300 grm. t.; 15 mm. v.h. = 450 grm. t.; 20 mm. v.h. = 610 grm. t.; 25 mm. v.b. = 765 grm. t.; 30 = 915; 40 = 1210; 50 = 1500.

appropriate strength applied direct to the distal stump of the cut motor nerve). But the crest-height of the reflex contraction is attained later than that of the maximal twitch even when the height of the former is considerably less than that of the latter. The decline of the reflex contraction is considerably longer than that of the twitch. These differences accord with the considerations admirably given by Forbes and Gregg (3) in their discussion of their observations comparing the galvanometer records of the action current of the break-shock flexion-reflex (decerebrate) with that of the directly stimulated motor nerve. They pointed out that such differences as those above are not incompatible with the centrifugal impulses in the reflex, being, as for the motor twitch, not more than one, *i.e.*, not multiple, per nerve-fibre, since the total volley in the reflex may very probably be less nearly synchronous, *i.e.*, may, though single per fibre, be distributed through the constituent motor fibres as a group during a somewhat longer period of time.

Through a certain range of further increase of the break-shock stimulus the same above-mentioned characters of the reflex contraction as compared with maximal twitch continue to hold, but the crest-height of the reflex contraction continually approaches more and more to that of the maximal-twitch and the duration of the reflex contraction continues to be no less and usually still further prolonged. Then, as the stimulus is further augmented, there is reached a strength of stimulus at which the crest-height of the reflex contractions equals and, with strengths of stimulus beyond that, exceeds, the crest-height of the maximal twitch itself, while likewise the total duration of the reflex contraction exceeds, usually very considerably, that of the maximal twitch.

The strength of stimulus at which the reflex contraction thus begins to surpass the maximal twitch's crest-height is not necessarily great. Thus, in the experiment from which fig. 1 is taken, the reflex contraction's crest-height began to exceed that of the maximal motor-nerve twitch when the secondary coil stood at 45 cm. from the primary, the maximal twitch compared with it being sampled at 16 cm. of secondary's distance from primary. The stimulus for the reflex was, therefore, very weak relatively to that used for the maximal twitch, though the contraction evoked by the former was the greater. The reflex contraction evoked by the coil at 40 cm. (fig. 1), still much weaker than that used for the standard maximal twitch, exceeded the maximal twitch yet more. With further increase of the stimulus the tension developed by the reflex contraction may become considerably more than twice as great as that developed by the maximal twitch; and the duration of reflex contraction twice or thrice or even four times that of the twitch. In the final steps of increase of the stimulus, *e.g.*, where the break-shock can be felt when the electrodes are placed on the tongue, and at closer coil distances still becomes unpleasant (secondary coil at 16 cm. or less), the resulting increments in reflex contraction tend to be increments of duration more than of crest-height (figs. 2 and 7). With these strong stimuli the period of declining tension tends to be drawn out and to exhibit irregular undulations.

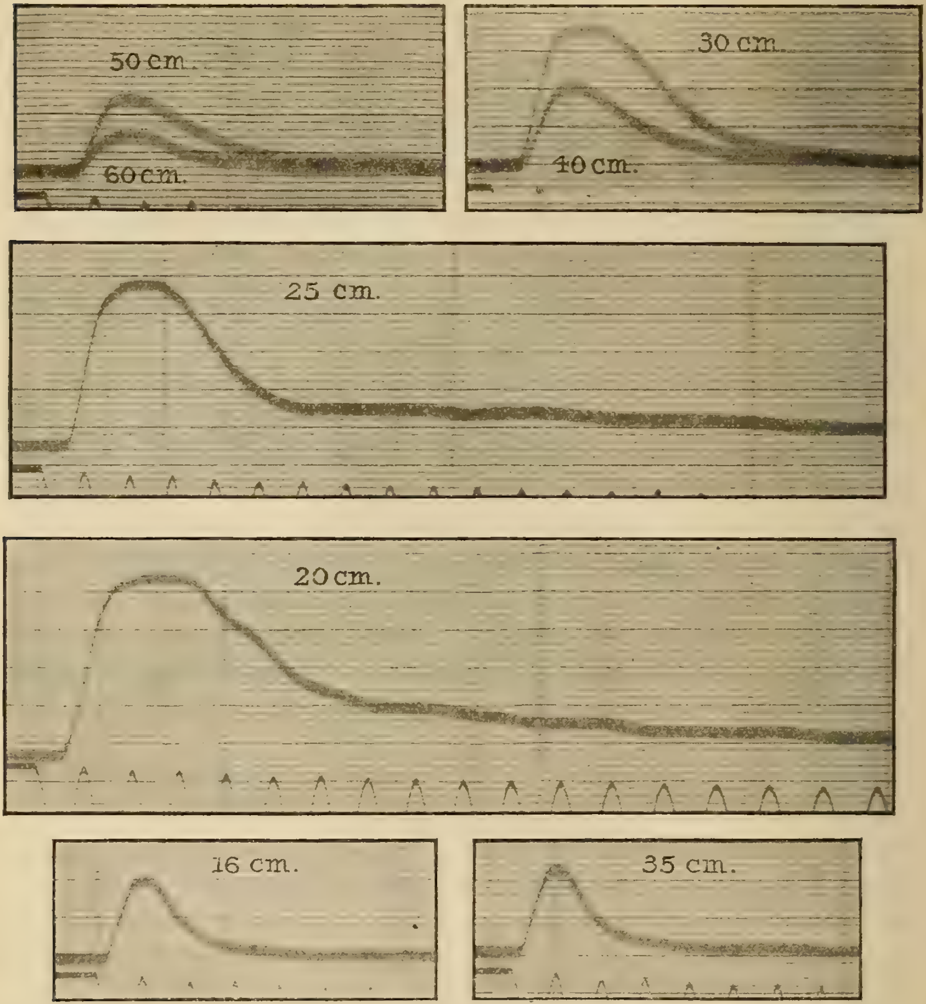

Fig. 2.—Similar to fig. 1, but the break-shock reflexes evoked from internal saphenous nerve instead of popliteal. The numerals at each inset give the coil distances for the break-shock. The two lowest insets show the maximal twitch elicited *viâ* motor nerve direct with the coil distances for the break-shock employed. Time ordinates and tension abscissæ as in fig. 1. Stimulus-signal below.

The above description applies to the spinal preparation as employed in

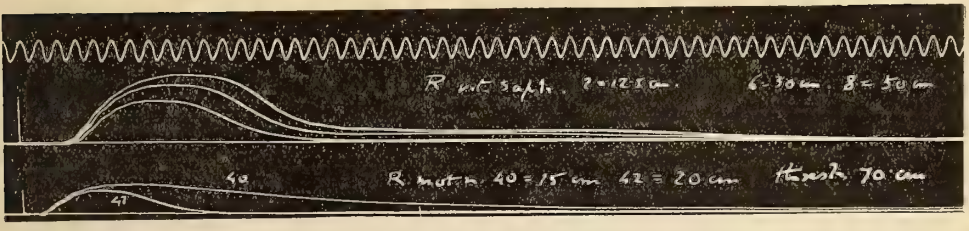

Fig. 3.—Upper curves. Three break-shock reflexes (tibialis anticus, spinal cat) from internal saphenous at coil distances marked on the figure. Lower curves, a maximal twitch by break-shock to motor nerve at 20 cm., and a "supramaximal response" by break-shock at 15 cm. coil distance. Time marked by 100 d.v. fork. Isometric "fall" myograph. Motor-nerve threshold at 70 cm.

these experiments, that is, at times varying from four hours to twenty-eight days after spinal transections at levels between the 9th thoracic and the 2nd lumbar segments of the cord. But, as shown previously (2), (7), (8), the description cannot be carried over with security to the decerebrate preparation. In this latter the reflex threshold is higher (7), (8), the reflex contraction is less strong and runs a different course (2), (7), and the latent period is, in my experience, considerably longer. These differences from the "spinal" reflex are instanced in the records given in fig. 5. The latencies are as long as some of those found by Jolly (11), with action currents, for the contra-lateral

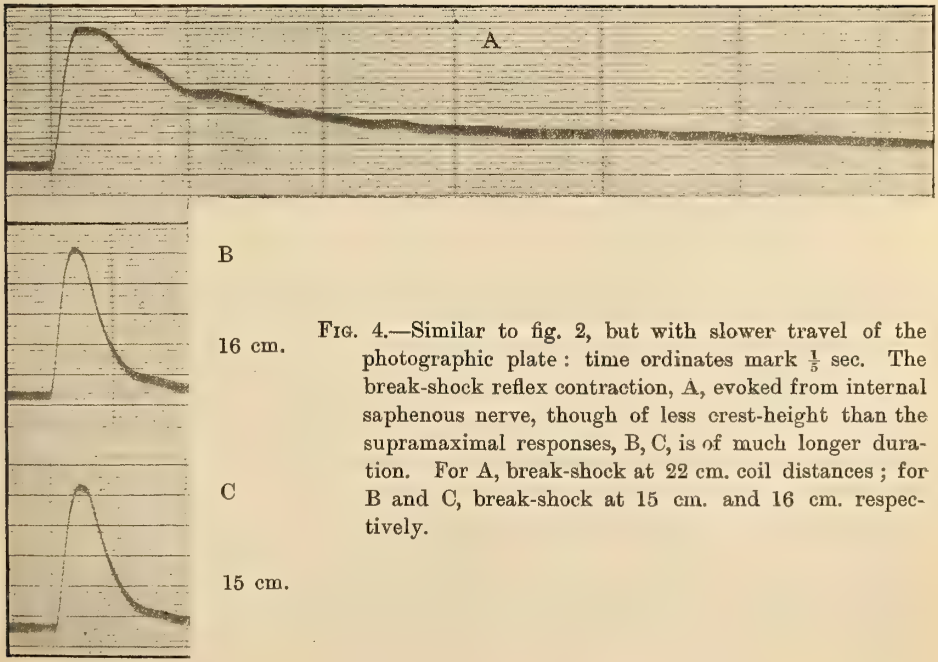

Fig. 4.—Similar to fig. 2, but with slower travel of the photographic plate: time ordinates mark $\frac{1}{5}$ sec. The break-shock reflex contraction, A, evoked from internal saphenous nerve, though of less crest-height than the supramaximal responses, B, C, is of much longer duration. For A, break-shock at 22 cm. coil distances; for B and C, break-shock at 15 cm. and 16 cm. respectively.

reflex. They and the course of the contraction suggest that in the decerebrate preparation the contraction of this ipsilateral reflex is possibly, in some cases, the rebound phase of a diphasic reaction, whose first phase is mainly inhibitory, owing to admixture of concurrent inhibition and contraction, although the inhibition cannot, owing to absence of actual contraction in the muscle at the time when the reflex is initiated, make itself apparent by any relaxation of the muscle, but only by delay and reduction of the ensuing contraction.

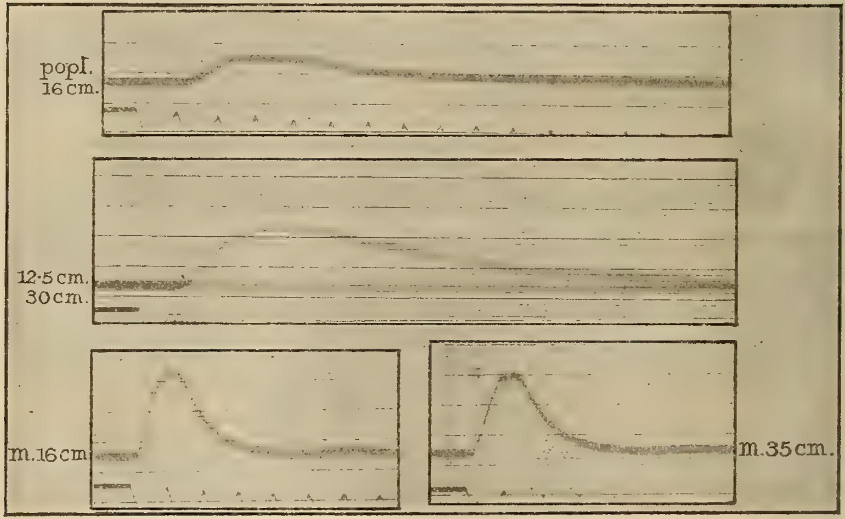

Fig. 5.—Similar to fig. 1, but from a decerebrate preparation instead of a spinal preparation. The upper two insets are reflex contractions (tibialis anticus) evoked by single break-shock to central stump of popliteal nerve; the numerals against each inset indicate the coil distances—the shocks for the reflex contractions are very strong; in the middle inset the break-shock at coil distance of 30 cm. produced no contraction. The lower two insets are maximal twitches evoked by break-shock to motor nerve at 16 cm. and 35 cm. respectively. Time ordinates and tension abscissæ as in fig. 1. Stimulus-signal below.

The observations, in so far as they cover the ground of those reported in the two previous papers (1 and 2) confirm and amplify them. (1) A single break-shock of moderate intensity, applied to a bared afferent nerve, not rarely evokes a reflex contraction exceeding in tension and duration the "maximal" twitch elicitable by break-shock stimulation of the motor nerve itself. (2) The rise of tension in the reflex contraction very soon after its first onset is not rarely of steeper gradient than is that of maximal twitch. This steeper gradient is observable in some instances even before the termination of the first 0·01″ from commencement of the contraction. (3) The maximal tension of the reflex contraction is attained later than that of the twitch. (4) The crest of the reflex myogram tends commonly to be more plateau-like than is that of the twitch.* (5) The decline of tension of the reflex contraction is more prolonged than is that of the twitch. (6) The reflex myogram elicited by the break-shock *vià* each of the afferent nerves used is of more or less characteristic form for each of those nerves severally. Thus, internal saphenous gives a lower and more prolonged crest-plateau (figs. 2, 3) than does popliteal.

These differences observable between break-shock reflex contraction and the maximal twitch evoked from motor nerve suggest that, while in the latter the motor nerve conveys but a single nervous impulse per fibre, in the former the centrifugal nervous impulses are, at least in some of the efferent fibres, repetitive. It was shown by Sassa and myself (2) that this difference persists after the proprioceptive reflex arc of the reacting muscle itself is broken. The responsible difference, therefore, between the nervous preparations compared for reflex and twitch is that while that of the latter consists of motor nerve, muscle, and neuromyal junction, that of the former comprises in addition the reflex (spinal) centre and the central stump of the afferent nerve. The added factor that at once appeals as that most probably responsible for the difference between the reactions of the two preparations is doubtless the spinal centre present in the former and absent from the latter. Prior, however, to accepting this inference, certain contingencies have to be borne in mind.

In the mammalian motor nerve-muscle preparation, as in that of the frog, the muscle response under progressively increasing break-shock stimuli, starting from threshold value upwards, is a twitch contraction, which progressively increases in strength through a certain range of stimulus increments (sub-maximal), and then remains practically without further increase (fig. 6), *i.e.*, is "maximal" throughout a very considerable succeeding range of further increments of stimulus. When, however, this long range of maximal stimuli is followed to its stronger end, there comes a point where, with further increase of the break-shock, the muscular response begins again to increase, and increases rapidly (fig. 6). This point in the inductorium used in these experiments showed considerable variation, but was usually where secondary coil was about 15 cm. on the scale from primary.

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* In view of the precautions taken to minimise lag and overthrow in the myograph used in these records, it is noteworthy that the crest of the motor twitch contraction in this mammalian muscle with intact blood circulation appears in the myograms not as a round-curved peak, but rather as a plateau, although the plateau is short, ending slightly more abruptly than it began.

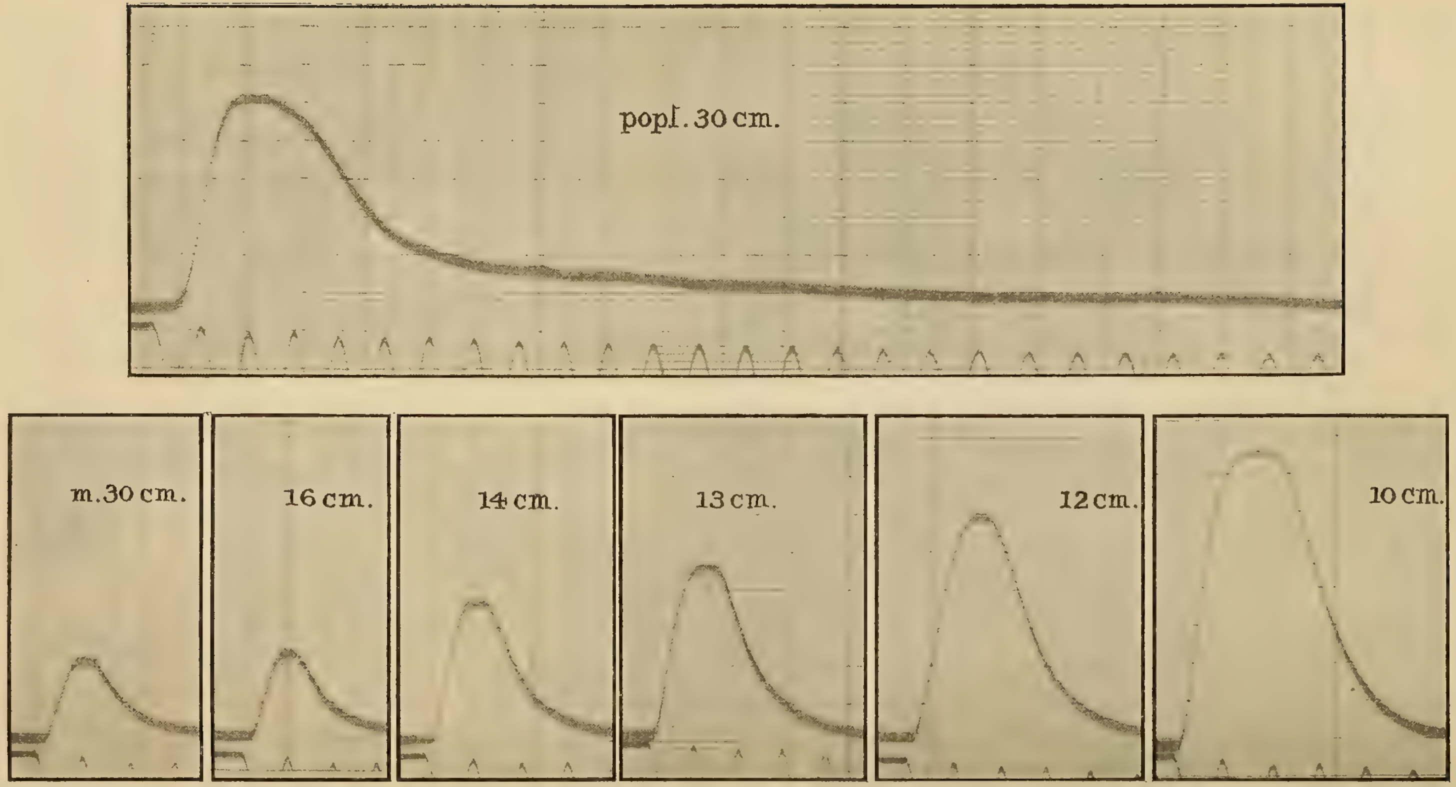

Fig. 6.—Lower row are maximal twitches and supramaximal contraction responses, evoked by single break-shock applied direct to cut motor nerve, for comparison with reflex contraction (upper inset) to single break-shock applied to central stump of popliteal nerve. Numerals against each inset give the coil distances for the break-shocks. The photographic plate was, as shown by the ordinates, travelling rather more quickly for the reflex record than for the other records, the speed for all these latter was the same. Tension abscissæ as usual. Stimulus-signal below.

Sometimes it did not occur even when 10 cm. was reached. These "supra-maximal" contractions thus elicited from motor nerve appear from their myogram features to be tetanic (fig. 6). They differ from the twitch in their greater height and the greater steepness of up-gradient which their ascent soon after its outset assumes, and, from the reflex contractions, in their brief latency, their more peaked, less plateau-topped climax and more rapidly falling, less prolonged, decline. Many of them cannot be explained by mere addition to the kathodal (closing) twitch of a simple opening twitch from anode, even supposing the intervention of the refractory phase of the nerve-fibre did not disallow such an assumption. Their course and character invite further examination, which I purpose to make. Their interest here is for the indications they afford that a single break-shock stimulus can, if of high strength, excite not merely one impulse, but a repetitive series of impulses in fibres of a motor nerve (*cf.* Forbes and Gregg (4)).

The form of the "supra-maximal" responses, as they progressively increase under successive increments of the break-shock stimulus, seems inadmissible of other interpretation. As the secondary coil is by equal steps brought nearer to the primary, the crest-height of the responses increases more and more rapidly, as does, of course, the physical value of the induced current. The development of contraction-tension thus reached by the higher members of the "supra-maximal" response series may amount to more than double that of the ordinary maximal twitch. This seems explicable only by their contraction being tetanic in nature. With the higher members of the series, the latent period between stimulus and commencement of contraction becomes remarkably shorter than that of the ordinary maximal twitch, though the place of the electrodes be retained for both at the same distance up the motor nerve. This led me to suspect at first that the strong current employed might by escape be exciting the muscle itself directly, although the distance between the muscle and the electrode the nearer to it was more than 60 mm. But on ligating, with a thread soaked in normal saline, the nerve 20 mm. distal to the electrodes, *i.e.*, between muscle and the seat of the electrodes on the nerve, all contraction in response to the strongest break-shock given by the electrodes at once ceased. Moreover, strong break-shocks applied to the afferent nerve show similarly no sign of "escape," as evidenced by the latency of reaction being still as great as with the weaker (fig. 5).

It is difficult, in the light of Adrian's demonstration that the "all-or-none" principle obtains in the impulse reaction of motor nerve-fibres, to suppose that these strong stimuli given to the motor nerve can cause it to deliver *stronger* nerve impulses to the neuro-myal junction or the muscle-fibres than do the moderate or weak. And since break-shock stimuli of lower strength than these strong ones already produce the maximal twitch, *i.e.*, are by definition "maximal" and excite all the motor nerve-fibres to the muscle, these latter strong ones cannot excite a greater number of the motor nerve-fibres than do those former. The seat of the initial causation of the "supra-maximal" response seems, therefore, excluded from being either at the neuro-myal junction or in the muscular fibres proper.

Bearing in mind, however, features of the "local excitatory process," the process recognised by Adrian and Keith Lucas (5), (6), to occur at the seat of stimulation of an excitable tissue, and elucidated by them, there seems no inherent unlikelihood that a single shock applied to a nerve should, if strong, evoke a short series of impulses in individual nerve-fibres. Forbes and Gregg (4) have already called attention to this probability. Persistence of the excited state at the locus of application of the strong stimulus should, if suitably prolonged, result in initiation thence of a succession of propagated disturbances, impulses, along the fibres. This view merely demands that the nerve-fibre reacts essentially similarly to cardiac muscle-fibre. Garten (9) has shown that under continued stimulation by a strong voltaic current the response of the mammalian nerve is a rhythmic series of action-currents.

But if motor nerve-fibres can be excited by a single induction shock so as to react repetitively, so also presumably can afferent nerve-fibres. The evidence of repetitive discharge in the break-shock reflex may, therefore, be referable to repetitive response on the part of the directly stimulated afferent fibres themselves rather than to repetitive response developed in the reflex centre. To this possibility the present observations supply no entirely decisive answer. It is noteworthy, however, that the motor nerve-fibres indicate tetanic response only when the break-shock stimulus is of a far higher strength than are break-shocks fully sufficing to evoke reflex contractions of tetanic character when applied to the afferent nerve. These strong break-shock stimuli are presumably of a strength corresponding with that named by Forbes and Gregg (4), in their work with action-currents, "the maximal limiting value." If the tetanic character of the reflex contraction is attributable to repetitive response in the afferent nerve-fibres themselves, these latter must differ from motor nerve-fibres by reacting repetitively to stimuli sometimes a hundred-fold weaker than those required for making ordinary motor nerve-fibres so react. Again, the differences (figs. 4 and 8A) are striking between the myogram forms of, on the one hand the "supra-maximal responses" produced by break-shock stimulations of motor nerve, and on the other hand the break-shock reflex contractions evoked by stimulation of the afferent nerve. Even in instances wherein the two crest-heights are approximately equal, the time-relations of the development and subsidence of tension are widely different. In no break-shock reflex have I met decline of tension in relation to crest-height so rapidly precipitous as it is quite commonly in the "supra-maximal" motor-nerve responses. Again, on the view that the tetanic character of the reflex contraction evoked by a break-shock applied to the afferent nerve is referable to repetitive reaction to the break-shock by the stimulated afferent nerve-fibres themselves, the distribution of the repetitive process both in time and among the individual nerve-fibres must be assumed to be considerably different in the internal saphenous nerve from what it is in the popliteal nerve; the low prolonged break-shock reflex from the former contrasts with the higher less plateau-like fountain-topped reflex contraction given by the latter. Again, the fact that the intensity and extent of the break-shock reflex contraction is much greater in the spinal preparation than in the decerebrate, a circumstance which affects the conditions of the spinal centre but cannot be thought to influence that of the afferent nerve-trunk itself at the seat of stimulation, argues for the centre rather than the afferent nerve being responsible for the development of the tetanus of the reflex response.

On the other hand, if the motor nerve-fibres begin to respond repetitively at high strength of the break-shock stimulus, it is prudent to suppose that so also do the afferent fibres under similar strong stimuli. In this the results endorse the opinion reached by Forbes and Gregg (4), who write after their study of the nerve action-current, and their experience of the spread of reflex activity to remote muscles in the decerebrate preparation: "Of the conceivable explanations" . . "that have occurred to us, we incline to regard as the most probable that a second propagated disturbance, or even a series of them, may be evoked in each afferent nerve-fibre by a single shock, if sufficiently strong."

Evidence of this may be looked for, in the present experiments, in some change possibly discernible in the character of the reflex contraction on and after the break-shock reaches about that strength at which it excites "supra-maximal" contractions when applied direct to the motor nerve, *i.e.*, at about 15 cm. on the scale of the inductorium used in these experiments. A change in the form of the reflex myograms is in fact noticeable usually at about this place on the inductorium scale. This change consists, as mentioned earlier in the text, in a considerable further prolongation (fig. 7) in the duration of the reflex contraction to the break-shock, accompanied by little or no further increase in the crest-height, the reflex in its slow subsidence not rarely exhibiting late subsidiary crests (fig. 4), assuming a somewhat dicrotic or even tricrotic character.

That a reflex contraction is sometimes of the nature of a simple twitch, *i.e.*, non-tetanic, seems clearly shown by observations of Jolly (10) and of Wertheim-Salamonsen (12) on the muscular action-current in the knee-jerk. But the probability seems that the tetanic character so commonly observed in the reflex contractions dealt with in this paper, as evoked by a break-shock applied to the bared afferent nerve, is, when that shock is of weak or moderate intensity, referable to the reaction of the centre itself, and is, even when the break-shock is stronger, still chiefly due to the centre. That is to say, even when initiated by so brief a stimulus as a single induction shock, the reflex action seems to involve some central process whose result is repetitive discharge of impulses. The repetitive discharge of motor impulses is obviously not to be thought of as more than the final outcome of the reaction in the "centre." The relatively prolonged duration of that outcome and the long central latency preliminary to it, together with other more general considerations, suggest that underlying the discharge there is some central process of other kind; this underlying process need not, from the repetitive nature of the discharge, be inferred to be itself repetitive, or if under some circumstances rhythmically maintained, to be itself essentially rhythmic. It finds expression through a mechanism (nerve-fibre) whose only mode of response

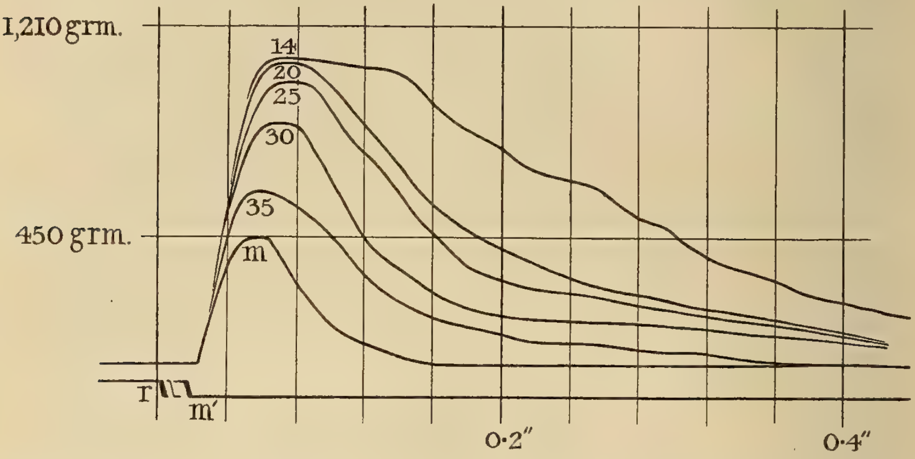

Fig. 7.—Superposed tracings of photographic myograms all from same experiment comparing those of greater crest-height than the maximal twitch, and indicating the change in the height and duration relation when the break stimulation to the afferent nerve (poplit.), is pushed to high strength, *i.e.*, 14 cm. The lowest curve (*m*) is the maximal twitch as given from 35 cm. up to 20 cm. The numerals opposite the other curves give the coil-distance of the break-shock for each; tibialis anticus, spinal cat. Time ordinates mark $\frac{1}{25}$ sec.: the drop of signal line at *r* shows latency for the weaker reflexes, at *m′* the latency for the motor twitches; the faint line of drop between these gives latency for the strongest reflexes.

to excitation of more than very brief duration is rhythmic (tetanic). If, for the convenience of avoiding periphrasis in referring to it, we speak of the central process underlying and at back of the repetitive centrifugal discharge as the "charge" in contradistinction to the "discharge" it evokes, one feature that we may infer about the "charge" is its relatively long duration, *i.e.*, relatively to the whole cycle of a nervous impulse, and another is that, in response to

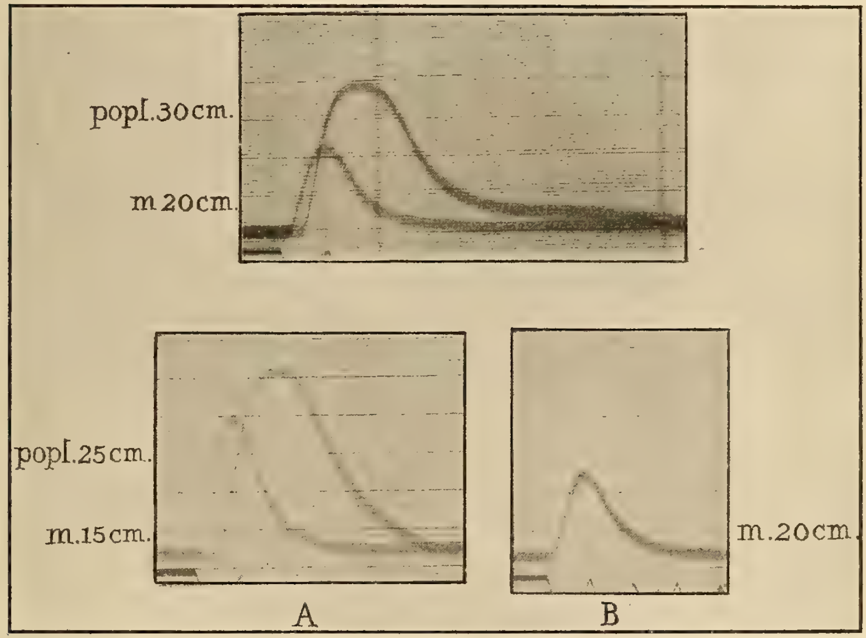

Fig. 8.—Upper record: reflex contraction of tibialis anticus (spinal cat) given by single break-shock at 30 cm. coil distance to central stump of popliteal nerve, followed on the same plate by record of maximal twitch given by single break-shock at 20 cm. coil distance, cut motor nerve; time ordinates at $\frac{1}{25}$ sec. intervals; tension abscissæ 31 grm. per millimetre. Lower record, A, similar to above from another experiment: the reflex by break-shock at 35 cm. coil distance, and the motor response distinctly "supramaximal" by break-shock at 15 cm.; the true maximal twitch, as determined by subsequent trial, is given at B, the instance shown being with break-shock at 20 cm. coil distance. Stimulus-signal below.

stronger break-shock stimuli of the afferent nerve, other conditions remaining the same, it attains a given degree of intensity, or amounts to a given quantity, more rapidly and dies out less soon than when such stimuli are weaker. In searching for a refractory phase for it in the flexion-reflex, Miss Sowton and myself (7) could find no positive evidence of such; that is to say, the period of refractoriness that was indicated for the reflex in our experiments was so brief that it could be accounted for apart from the central process by the refractory period of the afferent or efferent fibres themselves. In several respects it seems to me that the central "charge" process suggests comparison with the "local excitatory process" of Adrian and Keith Lucas, referred to above. In using for it the term central "charge" there is, of course, no intention of implying to it the electrical attributions of that term.

Summary.

The maximal twitch-contraction of tibialis anticus muscle (cat) evoked by a single break-shock applied to the cut motor nerve is compared with the same muscle's contraction as evoked reflexly (spinal preparation) by a single break-shock applied to an afferent nerve. The reflex contraction is found to exceed the former when the break-shock for the former is even considerably weaker than that employed for the latter. Evidence is given that this is due to the reflex response being tetanic in nature. If the break-shock is, however, quite strong (*i.e.*, above the limiting maximal value of Forbes and Gregg) there is evidence that it excites even when applied to the motor nerve a response of tetanic quality. The so-called "over-maximal twitch" is in reality a response of this kind. Such responses are in this paper termed "supra-maximal responses."

Probability is shown that a reaction of like kind obtains in the afferent nerve when the single-shock applied to it is of comparably high value. In this latter case there is added to the tetanic reaction of the spinal centre a tetanic reaction from afferent nerve fibres themselves. But with weak and moderate break-shock stimuli the seat of origination of the tetanic character of the reflex discharge appears to lie mainly, if not wholly, in the centre itself. It is inferred that it arises there from a process, a "charge" process, which is relatively long-lasting in comparison with the cycle of a nerve-impulse, a process which is more intense and of longer duration when the afferent fibres excited are many than when they are fewer.

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BIOLOGICAL SCIENCES.

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The Development of Vegetation in the English Lakes, considered in relation to the General Evolution of Glacial Lakes and Rock Basins.[*]

By W. H. Pearsall.

(Botany Department, University of Leeds.)

(Communicated by A. G. Tansley, F.R.S. Received April 28, 1921.)

Introduction.

During the past seven years, 1913–20, a large number of observations have been made in the English Lakes in an attempt to obtain some knowledge of the life conditions of aquatic plants. While the immediate object was to obtain light on the factors governing the distribution of these plants, it was also hoped that the results might bear upon more general problems of aquatic biology. The considerable data thus obtained, though far from complete, seem to be of interest from both these points of view. They are also capable of a more general interpretation, which is here attempted, and which, for the sake of clearness, may be indicated at this stage.

It is assumed that lakes and lake basins become modified as they increase in age. Evidence is then presented for considering the English Lakes as a series illustrating this process of lake development, and an attempt is made to describe the phases in this process, and to relate the marked differences in lake vegetation and fauna to the stages in the topographical evolution of a lake.

The data given below refer to the normal summer conditions of these lakes, and the methods employed in obtaining them have been described in a previous paper.[†] The arduous work of surveying the lake shores has only been possible through the unsparing assistance of my father, W. Harrison Pearsall, to whom I am very greatly indebted. The water analyses given were undertaken by Messrs. A. W. Richardson and R. Jaffé, to whom my thanks are due.

General Features of the Lakes and Lake Area.

The English Lakes lie among the high hills of Cumberland, Westmoreland, and North Lancashire. These hills consist essentially of a central uplift, from which run out ridges, radiating like the spokes of a wheel, and gradually decreasing in height as they pass further away from the centre. Practically all the main valleys between these ridges contain one or more lakes, varying greatly in size, but otherwise somewhat similar in appearance and characteristics. It is with eleven of the larger lakes that the present paper deals; Thirlmere, now practically a reservoir with variable water level, and those lakes smaller than Buttermere, having been omitted for the sake of uniformity. The remaining lakes vary in length from 10·5 miles (16·8 kilom.) (Windermere) to 1·26 miles (2 kilom.) (Buttermere), and have mean breadths of from 0·5 to 0·25 miles (0·8 to 0·4 kilom.). They lie at uniformly low altitudes, Hawes Water, 694 feet (211 m.), being the highest. Our knowledge of their chief sub-aqueous features is due to the work of Mill and Heawood,* from whom the table of their dimensions (Appendix I) is chiefly compiled.

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[*] The cost of this investigation has been defrayed in part by grants from the Royal Society.

[†] Pearsall, "The Aquatic Vegetation of the English Lakes," 'Journn. Ecol.,' vol. 8, 1920.

Origin of the Lakes.—As is well known, the whole of the Lake District was very heavily glaciated during the Ice Age, with the result that soil, surface deposits, and all but the boldest features of pre-glacial scenery, were removed by ice, leaving a bare and sterile rock mass, here and there overlaid by moraines and boulder clay. The effects of ice were particularly striking in the valleys. These were deepened and scoured out, the greatest erosion taking place towards the heads of the depressions, where the valleys (and the ice) were deepest. Thus, on the retreat of the ice, a number of *rock basins* were left, occupying the lines of the pre-glacial valleys, and tending to have straight steep sides, all minor points having been torn away by the ice and removed, along with any pre-glacial alluvial deposits (see Marr).†

The present lakes lie in these rock basins, and it may be noticed that the theory of their origin presupposes that their primitive condition was essentially *rocky*, a supposition borne out by the nature of the shores and islands at the present day. Glacial deposits also frequently exist, often rounded morainic boulders, and sometimes an impervious boulder clay. Neither the presence of these, nor the fact that they may hold up the lake to a higher level (as at Windermere and Bassenthwaite), invalidates the conception that these lakes are essentially rock basins, and are, in origin, of great similarity.

Modifying Agencies.—*Wave action* produces numerous well marked effects. Along the shores one can typically distinguish three chief zones caused by wave action: (1) the *gravel* wave-cut terrace just below the water level (2) a comparatively silt-free area of stone or boulder clay, often sprinkled with *sand;* (3) a silted (and more or less muddy) zone in deeper water. Generally, the stones or gravel of the terrace extend to about 2 m. in depth, while the finer sands and silts lie below 4 or 5 m. The finest sediments tend to get carried along the shore by the littoral current, and they are deposited in bays or holes, where this current slows down as it passes into deeper water. It follows, therefore, that bays and holes tend to get silted up—points and exposed shores to be kept silt-free. Since the lakes are of different lengths and lie at different angles to the prevalent winds (here S.W.), the strength of wave action varies very greatly, and its results are shown in very different degrees. For instance, wave effects are most severe in Wastwater, Coniston, Windermere and Ullswater, all long lakes lying roughly S.W. to N.E.

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* 'Geogr. Journ.' (London), vol. 6, 1895.

† 'The Geology of the Lake District,' Cambridge, 1916.

A second type of modification is produced in any lake by the presence of *fluvial* sediments. The lateral affluents of lakes lying in such steep-sided valleys as these are normally rapid, and carry down into the lakes quantities of coarse detritus, chiefly gravel. The mouth of every small stream thus, in time, projects into the lake on a gravel delta, and as a lake increases in age, its shores tend to become more and more irregular owing to the development of these deltas. It then follows that parts of the shores become sheltered and more silted than they otherwise would be.

This modification of the lake shores is only one sign of the effects of regional erosion and this group of factors produces other changes in the characters of the lakes. As the surrounding country weathers from crag and scree into gentle slopes and soil, the gradients of the streams tend also to decrease and their carrying power is diminished, particularly where they enter the lake. Hence, they ultimately carry into the lakes, not gravels but *silts*, and since the surrounding country is developing soil as it becomes more eroded, *finer silts tend to become available in increasing quantities*, replacing the sands and gravels of the earlier stages of lake development.

Geology of the District.—As the map shows, three main types of strata exist —to the north, the Skiddaw slates; in the centre, the volcanic ashes and lavas of the Borrowdale series; and in the south, a group of Silurian slates, grits and flags. In their appearance and general characters, these three types of rocks are very similar, but though all are hard, the central volcanic rocks are harder than the sedimentary rocks of the Silurian and Skiddaw beds, and, moreover, possess larger and more regular joints. Hence they weather more slowly, and also give rise to block screes, rather than to finer products. The effect of this upon the topography of the district is very noticeable. Having weathered least, the central hills tend to be higher, more rocky and steeper, while around them lie the lower and more eroded hills of the softer strata, usually round-topped and covered more or less with soil. So, too, the lakes on or near the central harder mass tend to be more rocky, while on the softer strata they are more silted. Some of the bolder slopes and the more rocky lakes may, however, lie on the Skiddaw slates (*e.g.*, Buttermere, Crummock and Derwentwater) and hence, it seems clear that it is *the degree of erosion of the surrounding country which largely governs the present physical condition of the lakes.*

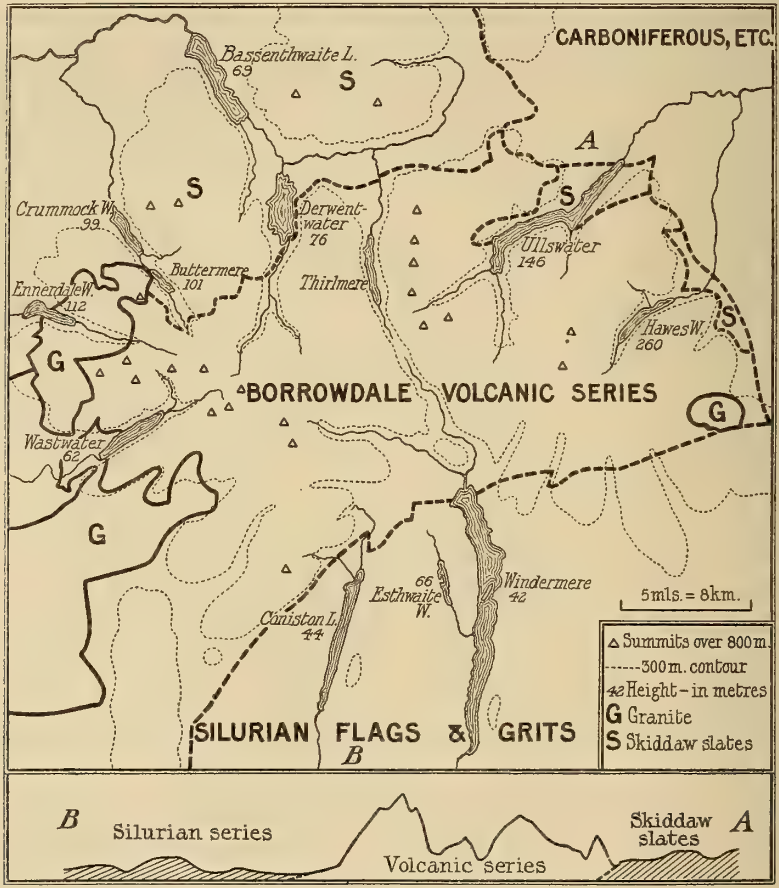
CARBONIFEROUS, ETC.
Bassenthwaite L.
69
S
A
S
Crummock W.
99
Derwent-water
76
S
Ullswater
146
Buttermere
101
Thirlmere
Ennerdale W.
112
G
Hawes W.
260
S
BORROWDALE VOLCANIC SERIES
G
Wastwater
62
G
66
Esthwaite W.
Windermere
42
5mls. = 8km.
Coniston L.
44
△ Summits over 800 m.
------300 m. contour
42 Height - in metres
G Granite
S Skiddaw slates
SILURIAN FLAGS & GRITS
B
B
Silurian series
Volcanic series
Skiddaw slates
A

Fig. 1.—Map of the English Lake District, with section showing the relation of scenery and gradients to the underlying rocks.

An endeavour has been made to express numerically the differences arising from these conditions of erosion, the results being included in Table I. As an expression of the types of slopes and soil-covering of the surrounding country, the percentage of cultivated land and alluvial pasture is given for the drainage system of each lake. The steeper slopes are either screes or rough pasture, the flatter hill tops are usually moorland. Only alluvia and the gentler soil-covered slopes can be cultivated, and hence the above ratio, though rough, is a fairly accurate expression of the character of the drainage system. From the figures given, it will be seen that the lake drainage systems fall into two classes, characterised by low and high proportions of cultivable land respectively.

Table I.—Effects of Erosion.

Lake.	Percentage of drainage system cultivable.	Lake shore to 30 feet depth. Per cent. rocky.	Relative transparency of lake water. m.
Wastwater	5·2	73	9
Ennerdale	5·4	66	8·3
Buttermere	6·0	50	8·0
Crummock	8·0	47	8·0
Hawes Water	7·7	25	5·8
Derwentwater	10·0	33	5·5
Bassenthwaite	29·4	29	2·2
Coniston	21·8	27	5·4
Windermere	29·4	28	5·5
Ullswater	16·6	28	5·4
Esthwaite	45·4	12	3·1

The second column in this Table shows the approximate proportion of the shore-line of each lake, which is rocky. From the soundings along the shores the average depth to which stones or rock extend has been determined, the deeper part of the shores being silted and soft. Taking 30 feet (9·2 m.) as a maximum, the percentage of the shore-line which is rocky can thus be obtained very approximately for each lake.

As the soundings on which these are based were made chiefly in bays, the figures given are possibly too small. They are, however, truly comparable. While the small size of Esthwaite may be the cause of the silted nature of its shores, as compared with Coniston, Windermere, and Ullswater, it cannot be assumed that the high degree of silting in the latter lakes is due to the slight effects of wave action, for Derwentwater, both smaller and with more irregular shores, has more rocky shore-lines. Similarly, Wastwater, in which the degree of wave action is almost the same as in Windermere, has shores which are much less silted. By comparison with the first column in the Table it will be seen that the *rocky* lakes (Wastwater to Crummock) lie among steep slopes and uncultivated land, the *silted* lakes (Derwentwater to Esthwaite) lie among the gentler soil-covered slopes. It seems a fair assumption, then, that the character of the shore-line, whether silted or rocky, depends largely upon the character of the surrounding country; but it should also be remembered that the softer rocks along the shores of the more silted lakes are more easily eroded by waves.

Another criterion of the silting conditions in any lake may be employed. Since suspended matter makes water more opaque, the relative transparency of the lake waters may be used as an indication of the quantity of suspended matter they contain. The basis of the comparison is taken as the greatest depth at which a white disc (7 cm. in diameter) can be seen. The results are contained in the third column of Table I, and they show that the clearest waters occur in those lakes with rocky shores. The test must be used with caution, for some of the lake waters are coloured with dissolved peaty matter, *e.g.*, Bassenthwaite and Esthwaite. But it is permissible to conclude from these results that there are greater quantities of suspended matter in the waters of those lakes with silted shores. Moreover, since only the finest matter can remain long in suspension, the greater opacity of the waters of these lakes indicates that their affluents bring in material *in a finer state of subdivision than that carried into rocky lakes.* It has already been shown that this is *a priori* probable. Further evidence in support of this assumption is obtained by comparing the mechanical analyses of muds taken from Wastwater, the most rocky lake, with those taken from Esthwaite, the most silted lake. The following results are then obtained:—

Table II.—Mud Analyses.

Material.	Wastwater (rocky). Per cent.			Esthwaite (silted). Per cent.			
Sand	75·1	82·8	74·9	62·0	54·2	34·2	28·2
Silt	14·8	15·0	16·1	17·6	14·6	29·3	25·3
Fine silt and clay	10·1	2·2	9·0	20·4	31·2	36·5	46·5
	1	2	3	4	5	6	7

The muds for these analyses were all obtained from depths of 8 to 12 feet (2·4 to 3·6 m.), so that they are fairly representative. The varying forces of wave action make an exact comparison very difficult, but the differences are sufficiently striking to make it probable that the sediments of *rocky* lakes tend to be *coarse,* while those of *silted* lakes tend to be *finer.* Field notes are quite in agreement with this conclusion.

Thus on their physical characters alone the lakes can be divided into two extreme types, *rocky* and *silted.* Wastwater, Ennerdale, Buttermere, and Crummock represent the *rocky* type, while Coniston, Windermere, Ullswater, and especially Esthwaite, belong to the *silted* type. Derwentwater, Bassenthwaite, and Hawes Water remain as intermediate examples between the two extremes. Other attributes of these lakes will now be considered.

Chemical Characters of the Lake Waters.

Chemically the waters of these lakes possess much in common (see Appendix II). They are notably poor in dissolved minerals, and particularly in calcium and carbonates. Other ions, therefore, constitute an unusually large proportion of the dissolved matter, particularly those of the alkalies (Na and K), an unusual feature in pure natural waters. The sulphate-content is high in the case of Crummock, Buttermere, Bassenthwaite, and Esthwaite, the only lakes where drainage systems lie entirely on the Skiddaw and Silurian rocks. In other respects, the influence of various strata is not very apparent and the only satisfactory classification is obtained by arranging the lakes in the two groups, *rocky* and *silted*, as already distinguished. It then appears that:—

(1) The waters of the rocky lakes have a high ratio of $\frac{Na_2O+K_2O}{CaO+MgO}$.

(2) The waters of silted lakes are characterised by larger quantities of nitrates, carbonates and silica.

(3) The latter waters tend also to possess larger proportions of lime and organic matter.

All the variations thus observed between the two types of water can apparently be related to the physical differences in the lake-basins and their drainage systems. The ratio $\frac{Na_2O+K_2O}{CaO+MgO}$ is chosen to represent the difference in the character of the bases. The tendency for the ratio to be low in silted lakes is probably in part due to the adsorption of K by sediments. The following facts bear out this assumption. While in Ennerdale and Wastwater potassium constitutes 50 to 60 per cent. of the whole K-Na residue, in Esthwaite and Ullswater it rarely exceeds 20 per cent. It is, moreover, well known that the drainage water from normal soils rarely contains much K, owing to the adsorption of this ion by the electro-negative soil colloids, and the same effect is produced when soil particles (*i.e.*, silts) are suspended in water containing traces of potassium. It has been shown already that the silted lakes are surrounded by greater areas of fertile soil, and possessed of greater quantities of suspended matter in their waters, and it may therefore be assumed that greater quantities of potassium are adsorbed by silts from the waters of these silted lakes, in comparison with those of rocky lakes. In addition, silted lakes possess *finer* sediments than rocky ones, and it is found that the finer silts contain larger proportions of potash than the coarser

(Table III). Finally, silts deposited from the lake waters are uniformly richer in potash than are the unaltered sands and glacial " clays " of the lake floors, *e.g.*, Nos. 2, 14, 16, Table III.

Table III.—Proportions of Potash.

No.	Sand.	Silt.	Fine silt.	K_2O.	No.	Sand.	Silt.	Fine silt.	K_2O.
5	34·2	29·3	36·5	0·0535	10	73·4	19·2	7·4	0·0290
7	69·2	14·6	21·2	0·0462	13	71·7	14·1	14·2	0·0260
23	44·9	29·1	26·0	0·0410	9	76·6	14·0	9·4	0·0232
20	51·5	28·4	20·1	0·0370	15	82·8	15·0	2·2	0·0232
6	72·0	7·6	20·4	0·0342	11	76·8	18·0	5·2	0·0228 *b*
8	64·2	23·6	12·2	0·0338	2	63·1	16·1	20·8	0·0220 *a*
19	70·9	14·1	15·1	0·0336	14	78·0	10·9	11·1	0·0210
12	65·3	24·5	10·2	0·0301	16	75·1	14·8	10·1	0·0202

Figures as percentages. *a*, **Boulder clay with slight silt;** *b*, **slight silt over stones.**

Hence it may be inferred that fine silts adsorb potassium from the lake waters, resulting in a decrease in the ratio $\frac{Na_2O+K_2O}{CaO+MgO}$ as the lake becomes more silted. It may be noted that the adsorption of K would be accompanied by a liberation of Ca and Mg into solution.

The increase of carbonates, nitrates and silica in the silted lake-waters is apparently due also to the effects of silting. The development of soil in the drainage system and of silts in the lakes may be assumed to be followed, in each case, by the development of vegetation. Organic matter deposited is then decayed under the aerobic conditions of the soil, forming CO_2 and nitrates. If the soils are rich in bases, complete decomposition results instead of the partial disorganisation which results in the formation of peat. Similarly sub-aqueous silts rich in potash are characterised by organic matter decaying rapidly and completely, while coarser potash-poor silts accumulate organic matter which is relatively undecayed. (Nitrates are formed in potash-rich silts.) It is apparently correct, therefore, to assume that the adsorption of potassium by silts results in the acceleration and completion of the processes of decay, and the formation of increased quantities of CO_2 and nitrates, the end products of these processes. Thus it follows that the silted lake waters contain greater quantities of carbonates, nitrates and usually of organic matter, and we can probably attribute their higher proportions of silica, calcium and magnesium, in part, to the solvent action of CO_2. (There is, of course, a resultant decrease in the $\frac{Na_2O+K_2O}{CaO+MgO}$ ratio.) Thus clearly the main differences between the lake waters are closely related to the degrees of silting of the lakes.

General Conclusion.—A conclusion of even more significance is now permissible. The fact that these lakes lie in glacial rock-basins entitles us to assume that at the close of the glacial period their condition was more uniformly rocky than it is to-day. Lakes lying on the hardest rocks have changed least, they are still rocky and *relatively primitive.* Lakes lying on softer strata are now more silted and therefore *more highly evolved.* It is thus possible to consider the group of lakes as a series illustrating the stages in *the post-glacial development of a typical rock basin lake,* a conception of great value, since it enables us to study the stages in development of a post-glacial fauna and flora.

On this assumption, Wastwater and Ennerdale are most primitive, and probably are not greatly altered from their immediate post-glacial condition. Windermere, Ullswater, and particularly Esthwaite, are most highly evolved, the remaining lakes exhibiting various intermediate stages, of which Derwentwater is perhaps the most valuable. In adopting this view, it must be admitted that the topography of the extremely evolved lake basins—notably of Esthwaite—was probably never so primitive as that of Wastwater. This, however, affects the *degree* of evolution only; it does not affect its *direction,* which, in lakes lying among similar rocks, and relatively uniform glacial deposits, must have proceeded along the same main lines.

We can now apply this theory of lake development to the distribution of the aquatic vegetation.

Vegetation.

The vegetation of a lake area falls conveniently into two main groups:—(1) attached plants, or *benthos;* (2) free-floating plants, or *plankton.*

Benthos.—The distribution of the characteristic deep-water plants in these lakes is shown in Table IV, the numbers given representing percentages of the total deep-water flora. For convenience, the species are divided into three groups:—

(A) Primitive plants: *Isoëtes* and *Characeæ.*
(B) Silt-requiring plants: *Juncus, Callitriche,* and *Potamogeton.*
(C) Plants of organic soils: *Myriophyllum,* etc.

Elodea can be placed in both (B) and (C).

As most of the soundings on which these figures are based were made in bays, the proportions given for plants in group B are probably too high, but the results are comparable, since the same error exists in each tabulation.

An analysis of the habitat conditions under which these plants live shows that their distribution depends upon the characters of the substratum rather

Table IV.—Plants Growing below Depths of 2 m.

The numbers represent percentage occurrences, *i.e.*, the ratios of total records of each species, to total records of all species (taken as 100).

	Wast-water.	Enner-dale.	Butter-mere.	Crum-mock.	Hawes Water.	Derwent-water.	Bassen-thwaite.	Coniston.	Winder-mere.	Ulls-water.	Esthwaite.
A. *Isoëtes lacustris* L.	49	35	40	48	5	31	42	34	9	34	2
Nitella opaca Ag. *N. flexilis* Ag.	36	46	40	26	65	42	3	9	40	15	26
Chara fragilis Desv.	—	2	—	—	6	1	—	—	3	—	2
Total	85	83	80	74	76	74	45	43	52	49	30
B. *Juncus bulbosus*, L., *f. fluitans*, Lam.	6	5	7	8	1	7	2	2	—	—	—
Callitriche intermedia, Hoffm.	2	1·5	3	5	4	1	21	7	0·5	—	1·5‡‡
Potamogeton pusillus, L.	0·5‡	0·5	1‡	2	2	1	3	15	3	10	6·5
P. perfoliatus, Wulf.	—	—	—	—	0·5	4	—	13	11	17	11
P. prælongus, Wulf.	—	—	—	—	—	1	—	2¶	14	8	1
Total *Potamogeton* spp.	0·5	0·5	1	2	5§	6	3	30	29††	35	25·5§§
Elodea canadensis, Mich.	—	—	—	—	—	—	—	—	9	—	10
Naias flexilis, R. and S.	—	—	—	—	—	—	—	—	—	—	21‖‖
C. *Myriophyllum* spp.*	6·5	10	9	8	13	2	11	6	4	14	2¶¶
Utricularia spp.	—	—	—	—	—	7‖	—	1**	—	—	—
Fontinalis antepyretica, L.	—	—	0·5	1	1	2	2	6	—	—	6
Other mosses	—	—	—	2	—	1	2	2	—	0·5	—
Ranunculus spp.†	—	—	—	—	—	—	12	2	1	2·5	—
Sparganium minimum, Fr.	—	—	—	—	—	—	2	1	1·5	—	4

* Chiefly *M. spicatum*, L.

† Probably *Ran. truncatus*, Koch.

‡ Including *P. polygonifolius*, Pourr., var. *pseudofluitans*, Syme.

§ Including *P. crispus*, L.

‖ Probably *U. major*, Sch.

¶ Also *P. alpinus*, Balb., and *P. zizii*, Koch.

** *U. intermedia*, Hayne.

†† *P. alpinus*, Balb., and *P. obtusifolius*, M. and K.

‡‡ *C. autumnalis*, L.

§§ Also *P. obtusifolius*, M. and K.

‖‖ *Naias*, 17 per cent.; *Hydrilla verticillata*, Casp., 4 per cent.

¶¶ *M. alterniflorum*, D.C.

than upon variations in the composition of lake waters or in the physical conditions of the habitat.*

Table V summarises the soil types upon which the more important species are found:—

Table V.—Species and Soils.

Plant community.	No. of samples.	Fine silt and clay percentage.		"Available" potash percentage.		Rate of silting.
		Range.	Average.	Range.	Average.	
Isoëtes lacustris	4	5·2–20·8*	11·8	0·020–0·023	0·021	Very slow.
Juncus fluitans...	5	2·2–14·0	7·5	0·023–0·029	0·025	Moderate.
Callitriche intermedia	3	7·4–16·5	12·6	—	—	„
Potamogeton perfoliatus	3	10·2–20–4	15·2	0·030–0·034	0·033	„
P. pusillus and *P. prælongus*	6	12·2–36·8	22·5	0·034–0·053 (4)	0·042	Rapid.
*Naias flexilis** ...	—	36·5 and 46·5	41·6	0·054	—	„

* A little altered boulder clay.

It will be seen that *Isoëtes* and *Juncus fluitans* occur on the coarser soils, poor in available potash, while *Potamogeton* and *Naias* are found on the finer and richer silts. The decrease in abundance of *Isoëtes* and *Juncus* in evolved lakes can therefore be attributed to the development of *Potamogeton* spp., consequent upon the increased abundance of finer and richer sediments.

It has further been shown that the development of silting on a lake shore leads to the replacement of *Isoëtes* by *Nitella,* which requires an easily penetrable substratum. Where sediments are abundant and the mud deep, higher plants (of group B) replace *Nitella.** The gradual decrease of the primitive plants from 85 to 30 per cent. in the lake series is due, therefore, to the silting up of the shores in the evolved lakes, and to the greater rate of sedimentation.

It may be noticed, finally, that, including *Elodea,* the plants of the more organic soils become more abundant and more varied in evolved lakes, which have presumably been colonised by plants for the greatest period of time.

The peculiarities of individual lakes may now be considered, to explain the variations in Table IV. The frequencies given for any species are based on the total records for the lake, reduced to the same relative proportions of the lake flora as in Table IV.

The Development of Vegetation (fig. 2).—In Wastwater and Ennerdale, both *Isoëtes* and *Nitella* are most often found at a depth of about 6 m., and have similar ranges of depth. The plants are normally rather scattered, and obviously colonising bare areas. Very large parts of the lake shores are devoid of vegetation. The depth distribution of *Isoëtes* and *Nitella* is remarkable when it is considered that, while the light limit for vegetation in these two lakes must be at least 10 m., probably more, yet little vegetation occurs below 7 m., or, normally, above 3 m. The development of vegetation between these two depths is undoubtedly due to the fact that wave-formed sediments are deposited more commonly at 4 to 7 m., while above this zone rocks are found, and below it rocks or glacial till. In well sheltered bays or near streams, the zone of silts may be much more extensive, and there the vegetation is correspondingly more widely spread. On the abundant sands near stream mouths, *Juncus fluitans* replaces *Isoëtes* and *Nitella*, and *Littorella* may be found locally on wave-beaten gravel. Normally, however, there is little zonation of different types of vegetation.

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* Pearsall, *loc. cit.*

Great differences are apparent when the Derwentwater figures are examined. *Nitella* is still most abundant at the same depth, but its downward extension is curtailed by light conditions. It is becoming more abundant in shallow water and, as in Windermere and Ullswater, its upper limit coincides roughly with that of the mean silted area. Rocks are rarely found below 5 m. *Isoëtes* is now most abundant in much shallower water—2·3 to 3·6 m.—and has obviously been replaced at greater depths by the combined effects of silting and *Nitella* competition, while its further extension into shallow water is stopped by the development of the *Littorella* zone on marginal gravel and sand. It is, in fact, confined to the least eroded and least silted parts of the shores, *i.e.*, the most primitive. This is the usual type of distribution of *Isoëtes* and *Nitella* in these lakes, and it is *due to the physical conditions of the lake shores.* Where, as in Ennerdale, the moulding and silting of the shores has progressed but little, and the waters are very clear, one may find *Isoëtes* below *Nitella* as well as above, the *Nitella* coinciding with the zone of maximum silting. A similar less frequent example in Ennerdale gives:—

(1) *Littorella,* abundant on gravel and sand to 4 m.
(2) *Nitella,* abundant on deep fine sand and silt, 3 to 5 m.
(3) *Isoëtes* abundant on little altered glacial clay, 5 to 8 m.

Returning now to Derwentwater, here again the higher plants, *Juncus fluitans* and *P. perfoliatus,* are chiefly confined to shallow water on abundant sediments near stream mouths, the appearance of *P. perfoliatus* being attributed to the finer nature of the silts. Small quantities of *Potamogeton* are also appearing in deep water, replacing *Nitella.*

In Windermere all these processes have progressed much further, as silts are much more abundant. Owing to the great development of *Littorella* and *Nitella, Isoëtes* is relatively scarce, while *Juncus fluitans,* in its deep-water form, is quite absent. *P. perfoliatus* and *Elodea* are becoming abundant in the region formerly occupied by *Isoëtes* (*e.g.,* Derwentwater), while *P. prælongus* and *P. pusillus* are becoming abundant in the *Nitella* region. All of these changes are attributable to the increase in abundance and fineness of the silts.

In Ullswater the abundance of *Potamogeton* causes a very marked reduction in the quantity of *Nitella.* Here, again, *P. perfoliatus* is developing where the *Isoëtes* zone is becoming silted (*i.e.,* in bays). If the development of these lakes followed an exact sequence, *Isoëtes* should be nearly absent from Ullswater, as it is in Esthwaite; but an impenetrable glacial till is common in Ullswater, which, unless removed by wave action, prevents the extension of *Littorella, Potamogeton,* or *Nitella.* The persistence of *Isoëtes* on these primitive substrata is one of the features of Ullswater (and Coniston) and partly accounts for the abundance of that species. The point will be discussed in more detail below.

Esthwaite, for which no diagram is given, shows a further advance on the Windermere stage, *Isoëtes* being nearly absent, and *Nitella* encroaching on the *Littorella* zone to depths of 1 to 1·5 m. In Esthwaite other higher plants (particularly *Naias flexilis*) are numerous, to some extent replacing *Potamogeton.*

In primitive lakes higher plants only occur on abundant coarse fluvial sediments in rather shallow water, and they are confined to a few localities in each lake. Their development as deep-water plants along the shore-line only occurs when silts become abundant, and there is a marked tendency for the plants to progress into shallower water, already noticed for *Isoëtes* and *Nitella,* and clearly due to the increased silting of the shores. The case of *P. perfoliatus* is a good instance of this point (see fig. 2). It is worthy of notice that the first *Potamogeton* to appear in quantity is *P. perfoliatus,* which normally occurs on coarser and poorer silts than *P. prælongus* or *P. pusillus.*

The examples considered above make it apparent that the zonation of aquatic plants along a shore is an attribute of evolved lakes, and is, moreover, primarily dependent upon the condition of the substratum as determined by the silting factor. Were one alone of these lakes to be considered, it might well be assumed that zonation was due to light conditions, but such a conclusion is difficult to reconcile with the series described above. This is equally well shown when a series of lengths of shore in a single evolved lake is examined. Generally speaking, the variations described above would be found, provided conditions of exposure varied sufficiently. Thus, an exposed shore would show the Wastwater or Derwentwater stages, a sheltered one the Windermere or Ullswater condition, though the range of light intensities at different depths would be similar in each case.

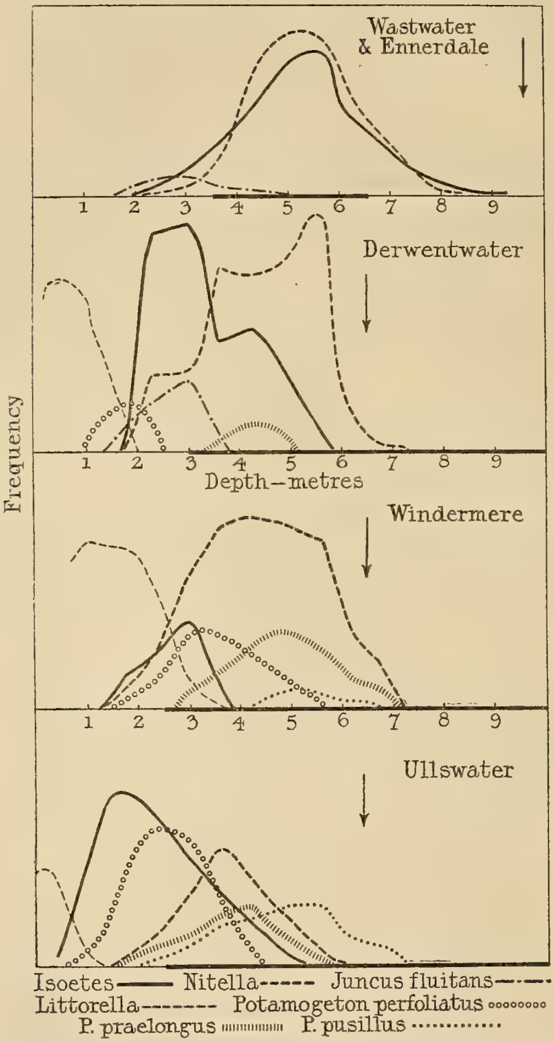

FIG. 2.—Depth distribution of characteristic species in the lakes named. The arrows show the light limit for vegetation. The thickened base line indicates the parts of the lake shores normally silted. The frequencies for any species are based on the total records for the lake, reduced to the relative proportions of Table IV.

Apparent Exceptions: Hawes Water.—This lake is apparently abnormal (i) in its high proportion of *Nitella*, *Isoëtes* being scarce, (ii) in the high percentage of its shore-line that is silted. It may be remarked that the second abnormality explains the first, since silting tends to replace *Isoëtes* by *Nitella*.

Both features can be accounted for by the fact that Hawes Water *has been heavily silted in the past,* shown not only by the condition of the lake floor but also by the size of the gravel alluvia at the mouths of the two main affluents. Moreover, in marked contrast to the gravel deltas in other lakes in this district, those at Hawes Water show no signs of recently deposited gravel. Mill (*loc. cit.*) also points out, quite justifiably, that the Measand Delta could never have been laid down by the present stream, and he attributes its formation to the "washing out" of a glacial lake. The third point of interest is that the sediments of Hawes Water are fine *sands,* not silts* such as would be carried into a lake by torrential streams. Very vigorous erosion in the past seems, therefore, to have washed huge quantities of sand and gravel into Hawes Water. The sand helped in the silting-up of the shores, it allowed the replacement of *Isoëtes* by *Nitella,* but it is too coarse to allow the extensive development of *Potamogeton,* spp. Thus Hawes Water, though abnormal, is not at variance with the views expressed previously.

Bassenthwaite owes its peculiarities to the brown and peaty nature of its waters, a coloration due to the extensive peaty alluvia at the lake head. The water is so discoloured that the lower light limit of vegetation is only 2·5 m., and the fine deeper water sediments cannot be colonised by plants. Owing to wave action, silting is naturally less rapid in shallow water, and moreover the sediments are coarser and become rapidly organic, owing to the slower deposition of silts, and their comparative poverty in potash. Thus, the vegetation of such a lake will be that characteristic (*a*) of wave-washed shores (little silted), (*b*) of the coarser sediments, (*c*) of the more organic sub-strata. The plants occurring in such habitats are (*a*) *Isoëtes,* (*b*) *Callitriche intermedia,* (*c*) *Myriophyllum* and *Ranunculus,* all of which are abundant in Bassenthwaite. The absence of the finer and softer sediments within the photic zone accounts for the scarcity of *Potamogeton* and *Nitella,* spp.

Coniston and *Ullswater* resemble Bassenthwaite in possessing a relatively high proportion of *Isoëtes,* yet their light conditions are normal and both include abundant *Potamogeton.* An examination of Table I shows that both lakes have a smaller percentage of cultivable land in their drainage systems than is compatible with the degree of silting, *i.e.*, their drainage systems are more primitive than the lake basins. It has already been remarked that their abundant silts are largely due to washings from lead or copper mines, and it is known, of relatively recent times. The coarser silts from such washings smother the lake floor round the mouth of the incoming streams, the finer are deposited all over the lake floor, *except in the regions subject to wave-wash, i.e.,* on most of the lake shores between the water margin and depths of 3 or 4 m. These wave-washed areas can only accumulate silt during calm weather, and hence change their character very slowly. Thus the discharge of ore washings into these lakes would cause an abundance of fine silts in deep water, and this would result in the replacing of *Nitella* by *Potamogeton* spp. On the other hand, the shallower water soils would remain little altered, and would retain their primitive vegetation of *Isoëtes.* Another factor tends to accentuate the retention of this primitive shore line in Ullswater and Coniston. The shores are relatively straight, as compared with Windermere and Derwentwater, and hence, offering no obstacles to the sweep of waves and littoral currents, are kept comparatively silt-free at depths of less than 3 to 4 m.

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* *Cf.* also Mill, *loc. cit.*

Shallow-Water Vegetation.—In the case of the shallow-water plants, much greater difficulty is experienced in making a comparative table of distribution. These plants are directly affected by wave action, and they are therefore very closely dependent upon the degree of exposure of the lake shores. In lakes like these, varying greatly in size, orientation and shelter, a direct numerical comparison is therefore impossible, and only the presence or absence of typical plant communities can be given :—

Table VI.—Distribution of Shallow-water Plants.

	Wastwater.	Ennerdale.	Buttermere.	Crummock.	Derwentwater.	Hawes Water.	Bassenthwaite.	Coniston.	Windermere.	Ullswater.	Esthwaite.
Littorella lacustris, L.	+	+	+	+	+	+	+	+	+	+	+
Potamogeton (submerged), spp.*					+				+	+	+
Myriophyllum spicatum, L.					+	+	+	+	+	+	+†
Ran. peltatus, Schr.							+	+	+	+	+
Potamogeton natans, L.‡				+	+	+	+	+	+	+	
Water-lilies§					+		+	+	+		+
Phragmites communis, Trin.				+	+	+	+	+	+	+	+
Scirpus lacustris, L.											
Equisetum limosum, L.		+	+	+	+	+	+	+	+	+	+
Carex inflata, Huds.											

* *Potamogeton gramineus*, L. (agg.), × *P. nitens*, Weber., *P. perfoliatus*, L., *P. alpinus*, Balb.

† *M. alterniflorum*, D.C.

‡ Including occasional *Castalia minor*, D.C.

§ *Nymphæa lutea*, L., and *Castalia alba*, Wood.

Even this rough method of comparison indicates that shallow-water plants are more typical of evolved than of primitive lakes, in spite of the considerable variations observed. Derwentwater, with a broken shore-line and numerous islands, is comparatively rich in this type of vegetation, and of the emergent species, *P. natans*, with the *Equisetum-Carex* type of reedswamp, predominate, though *Scirpus* and *Phragmites* are also abundant. The same plants are characteristic of Hawes Water and Bassenthwaite, though much less abundant. In Coniston and Windermere, *Scirpus* and *Phragmites* are more typical, along with some *P. natans*. In Esthwaite, water-lilies and *Phragmites* fringe most of the western shore. On the whole, as silting conditions improve, water-lilies and *Phragmites* tend to become more abundant in the lake series, a fact in agreement with their distribution on relatively inorganic soils, which are relatively richer in K_2O.* *P. natans*, *Equisetum* and *Carex inflata*, on the other hand, are found on highly organic soils, relatively poor in K_2O. Obviously, the increased development of finer and richer silts in evolved lakes tends to prevent the formation of these highly organic soils, except in land-locked and stagnant bays—which are uncommon in this lake area. It is therefore primarily to the silting factor that the development of both deep and shallow water vegetation is due.

General Conclusion.—This detailed discussion shows clearly that the fundamental factor in the development of the attached vegetation is the increase in abundance and richness of the sediments. Not only does this control the types of plant communities found, but it also limits the quantity of vegetation produced. Vegetation is sparse in the primitive lakes; it becomes increasingly abundant in the evolved lakes. As was suggested at the conclusion of the last section (p. 267), the lake sèries viewed in this way enables us to reconstruct the stages in the post-glacial development of these lakes. If this be done for the vegetation, we obtain the following means for the four main types of lake, and these represent four stages in the development of a post-glacial flora :—

Vegetation Types.

	Type of lake.			
	I. Primitive (4).	II. Intermediate (3).	III. Evolved (3).	IV. Esthwaite.
Primitive plants— *Isoëtes* and *Characeæ*	80·5	65	47·6	30
Juncus and *Callitriche* (requiring coarse silts)	9·4	11·9	3·1	—
Potamogeton spp. (requiring finer silts)	1·0	4·6	31·3	25·5
Naias, *Hydrilla* and *Elodea*	—	—	3·0	21·0

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* Pearsall, *loc. cit.*

Phytoplankton.—While no data are available for an accurate numerical comparison of the phytoplankton of these lakes, the investigations of W. and G. S. West* have already drawn attention to the fact that differences in its composition exist. The Wests' investigations included all the larger lakes except Coniston and Esthwaite. In addition, they described the periodicity of three lakes—Wastwater, Ennerdale and Windermere.† The following Table gives the maximum abundance of a few conspicuous plankton

Table VII.—Maximum Recorded Abundance of Principal Plankton Algæ.*

Wastwater	Desmids dt. (*Staurastrum jaculiferum*, West.); *Peridinium Willei*, Huit. Kaas and *Sphærocystis Schroeteri*, Chodat. ab.; *Rhizosolenia morsa*, West., and *Tabellaria spp.*, fr.
Ennerdale	Desmids dt. (*Staurastrum jaculiferum*, *lunatum* var. *planctonicum*, West, *longispinum*, Bail.); *Sphærocystis Schrœteri* and *Peridinum Willei* ab.; *Rhizosolenia morsa* fr.
Buttermere	Desmids dt. (*St. jaculiferum*); *P. Willei*, *S. Schrœteri* and *Dinobryon cylindricum*, Imhof. ab.; *Tabellaria* spp. fr.
Crummock	Desmids (*Staurastrum annatinum*, C. and W., *Spondylosum pulchrum* var. *planum*, Wolle) and *Dinobryon cylindricum* dt.; *Botryococcus Braunii*, Kutz., and *Cœlosphærium Kutzingianum*, Näg. ab.; *Tabellaria* spp., *Melosira granulata*, Ralfs, and *Sphærocystis Schroetiri* fr.
Hawes Water	Diatoms dt. (*Tabellaria* spp.); Desmids ab. (*St. jaculiferum*); *Cœlosphærium Kutzingianum*, *Sphærocystis Schrœteri*, *Eudorina Elegans*, Ehr., and *Ceratium hirundinella*, Müll. fr.
Derwentwater	Diatoms (*Tabellaria* and *Asterionella* spp.) and *Dinobryon* dt.; *P. Willei* and mixed Desmids ab.; *Eudorina elegans* fr.
Bassenthwaite	Diatoms dt. (*Tabellaria* and *Asterionella*); Desmids and *Ceratium hirundinella* fr.
Coniston	Diatoms (*Tabellaria* and *Asterionella*) and *Ceratium hirundinella* dt.; *P. Willei* ab.; Desmids fr.
Windermere	Diatoms dt. (*Asterionella*); Desmids (*Staurastrum paradoxum* Meyen), *Tabellaria* and *Ceratium hirundinella*, ab.; *P. Willei*, *Melosira granulata*, *Sphærocystis*, *Eudorina* and *Oscillatoria Aghardii*, Gomont, fr.
Ullswater	Diatoms dt. (*Asterionella*); *Tabellaria*, *Dinobryon* and *Chroococcus* spp. ab.; *Sphærocystis*, Desmids and *Ceratium hirundinella* fr.
Esthwaite	Diatoms (*Asterionella*) and Myxophyceæ (*Oscillatoria*, *Anabœna* spp.) dt.; *Tabellaria* ab.; *Ceratium*, *P. Willei*, Desmids and *Eudorina* fr.

* Frequency symbols: *dt.* = dominant, *ab.* = abundant, *fr.* = frequent.

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* "The Phytoplankton of the English Lake District," 'Naturalist' (London), 1909, p. 115.

† "Periodicity of Phytoplankton of some British Lakes," 'Journ. Linn. Soc.' (Bot.), vol. 40 (1912).

organisms, as recorded by the Wests or by the author. In view of the doubts expressed by some continental authors as to the reality of Desmid plankton—the percentage composition of the plankton is also given for August 23–30, 1920, when comparative samples were taken from all the lakes. These percentages are based on the number of individual organisms or colonies, *e.g.*, *Sphærocystis*, *Eudorina*, *Tabellaria*, etc.—the total number counted for each lake was over 1000. By comparison with other countings, these figures show rather low percentages of Desmids, and higher figures for Diatoms, except in the cases of Windermere, Ullswater, and Esthwaite. They are, however, the only figures available from collections taken at the same time.

Table VIII.—Percentage Composition of the Phytoplankton, August, 1920.

	Wastwater.	Ennerdale.	Buttermere.	Crummock.	Hawes Water.	Derwentwater.	Bassenthwaite.	Coniston.	Windermere.	Ullswater.	Esthwaite.
Desmids	*14*	*93*	*25*	*48*	19	7	1	4	11	5	7
Colonial Chlorophyceæ	*69**	2	*24**	*21*	10	4	0·5	9	8	1	5
Filamentous Chlorophyceæ†	7	2	18	2	1	4	6	4	6	5	1
Bacillariæ	9	1	9	1	*63*	*82*	*83*	*52*	*50*	*31*	5
Myxophyceæ	—	1	2	*23*	5	2	8·5	2	*19*	*30*	*64*
Dinobryon	—	—	13	3	—	1	—	—	2	27	14
Peridinieæ	1	1	9	1	2	—	1	26	4	1	4

* Almost entirely *Sphærocystis Schrœteri*, Chodat.
† Almost entirely Conjugatæ, especially sterile *Mougeotia*.

A comparison of these results fully justifies the conclusions that—

(1) The primitive lakes have a Desmid plankton in which *Peridinium Willei* and *Sphærocystis Schrœteri* are abundant, and Diatoms inconspicuous;

(2) The more evolved lakes have a Diatom plankton in which, on the whole, *Ceratium hirundinella* and *Eudorina elegans* are more typical;

(3) The three most advanced lakes have in addition a considerable element of Myxophyceæ, at times dominant in Esthwaite.

There is, therefore, a close similarity between the classification of the lakes on this basis, and that already made upon physical and chemical grounds. Since the characters of the plankton must depend very largely upon the salient features of the lake waters, such a marked similarity is to be expected, for it has already been shown that the properties of the lake waters are dependent upon the degree of physical evolution of the lake.

In the first place, the primitive lake waters are remarkable for their high $\frac{Na_2O + K_2O}{CaO + MgO}$ ratio, and only the waters of this type possess a predominant Desmid plankton. If it be assumed that the Desmid plankton requires such a high ratio of alkalies to other bases, it then becomes possible to explain not only the distribution of this type of plankton in this lake area, but also its presence in certain Scotch, Irish and Welsh lakes, for which data have been published. Moreover, the scarcity of Desmid plankton in European waters as a whole would then be due, on this assumption, to the scarcity of *pure* waters in which alkalies predominate (*i.e.*, excluding the sea and brackish waters). While it would lie outside the limits of the present paper to provide the data necessary to establish these statements, it may be assumed provisionally that Desmid plankton characterises waters with a high ratio of $\frac{Na_2O + K_2O}{CaO + MgO}$.

The abundance of Diatoms in the plankton of evolved lakes can also be correlated with the chemical characters of the lake waters. Since Diatoms have walls composed chiefly of silica,* they are obviously dependent on the presence of a certain amount of silica dissolved in the water. Thus a scarcity of silica—as in the primitive lakes—is accompanied by a scarcity of Diatoms, and these organisms are most abundant in those lakes containing the largest amounts of dissolved silica, *e.g.*, Windermere, Ullswater and Esthwaite. On the other hand a low $\frac{Na_2O + K_2O}{CaO + MgO}$ ratio is equally characteristic of fresh waters with a Diatom plankton and such waters are normally rich in nitrates. These factors may, therefore, be as necessary as the high proportions of silica.

A third element of marked interest in the plankton is the presence at times of abundant Myxophyceæ in Windermere, Ullswater and Esthwaite, well shown in the figures for August, 1920. It is noteworthy that these lakes have waters in which the organic content is normally *higher* than in the more primitive lakes, and it may be pointed out that Crummock Water has also a rather high organic content and a considerable element of Myxophyceæ. It is premature to say whether or no there is a *direct* connection between these facts, but it seems probable that they are in some way connected, as a dominance of Myxophyceæ is not unusual in waters having a high organic content.

A comparison of the bulk of the phytoplankton in these different types of lakes leads to the conclusion that *the greatest bulk occurs in the evolved lakes,* and that this is due primarily to the increased number of Diatoms (and Myxophyceæ). In this connection the results of Apstein,[*] taken with those of Brandt,[†] are of considerable interest. They show that in Holstein, lakes rich in nitrates contain the greatest bulk of plankton (chiefly Diatoms and Myxophyceæ), whilst scarcity of nitrates is accompanied by paucity of plankton. The water analyses show that this conclusion holds also for the English lakes, and it is therefore probable that Diatoms and Myxophyceæ are favoured by the presence of nitrates, provided their other habitat requirements are present.

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* Whipple and Jackson record the fact that 49·48 per cent. of the dry weight of *Asterionella* is silica ('Journ. New England Waterworks Assoc.,' vol. 14, 1899).

Since it has already been shown that the $\frac{\text{alkali}}{\text{CaO} + \text{MgO}}$ ratio, silica, nitrate and organic contents of the lake waters are dependent upon the physical condition of the lake basin and drainage system, there are strong grounds for the conclusion that the development of the phytoplankton, both in quality and in bulk, in any of these lakes, is directly due to the degree of development of the lake basin. W. and G. S. West regard contamination of the drainage system by cultivation and sewage as an important cause of plankton and Diatom abundance. While this recognises the effect of the probable increase of nitrates caused by habitations and cultivations, it is not in itself a fundamental distinction. As weathering and erosion change the topography of the lake basin, there is a constant increase in nitrification and change in the characters of the surface waters. Farms and cultivation *follow* the development of soil and gentle slopes. They perhaps accelerate or increase the developmental reaction; they do not inaugurate it, and, therefore, *the stage of evolution of the lake basin must be regarded as being the fundamental factor affecting the distribution of the phytoplankton,* since it is upon this factor that the characters of the waters depend.

Finally, according to the views laid down previously, we may regard these lakes as illustrating a post-glacial development of the phytoplankton. The succcession would then become :—

Desmids dt. *Peridinium Willei* ab. *Sphærocystis Schrœteri* ab. *Rhizosolenia morsa* fr.	→	Diatoms dt. (*Tabellaria* spp.) Desmids ab.	→	Diatoms dt. (*Asterionella*) *Eudorina* ab. *Ceratium* ab.	→	Diatoms (*Asterionella*) and Myxophyceæ dt.

Fish—Economic Significance of Results.

The economic importance of all limnological studies is largely bound up with their bearing on the problems of fresh-water fisheries. The following account of the Lake District fisheries is based on that of Watson,* supplemented and modified by the opinions of local fishermen and a long personal acquaintance with the fishing on some of these lakes.

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[*] 'Das Süsserwasserplankton,' Kiel und Leipzig, 1896.

[†] 'Wiss. Meeresunt.,' Kiel Komm., vol. 4, p. 215 (1899).

Fish are undoubtedly most abundant in the more evolved lakes and least so in the primitive ones; but this condition is due almost entirely to the numbers of coarse fish, perch (*Perca fluviatilis*), and pike (*Esox lucius*), which are especially abundant in Ullswater, Windermere, and Esthwaite, where they are increasing rapidly. The fish of the primitive lakes is undoubtedly the trout (*Salmo fario*), of which the largest numbers of well-grown fish probably occur in Crummock, Derwentwater, and Hawes Water. Pike and perch are absent from Wastwater, though the former were introduced some years ago without success. There are said to be a few perch in Ennerdale, but trout are the only abundant fish. On the other hand, trout are apparently decreasing rapidly in numbers in Ullswater and Windermere, and are practically extinct in Esthwaite. Char (*Salmo Willughbii*) occur in the deeper primitive lakes, and professional char-fishers say they also are decreasing in Windermere, as is known to be the case in Ullswater. Eels (*Anguilla vulgaris*) and minnows (*Leuciscus phoxinus*) are present in all the evolved lakes, and perhaps in Wastwater and Ennerdale also. It is certain, however, that there is a general progression in the lake series from the trout community to the perch-pike community, and the succession of these animal communities, therefore, closely parallels the development of the lake and its vegetation, as follows:—

Trout. Perch—Pike.
Primitive lakes. → Evolved lakes.

The economic value of fisheries may be assumed, roughly, to depend on two factors, the quantity of fish present, and their value from a commercial aspect, chiefly as food. From the former point of view, lakes like Windermere, Ullswater and Esthwaite rank first, since they undoubtedly contain the greatest numbers of fish per unit area. But, in food value, trout are the most important of the lake species, and, in this respect, the more primitive and intermediate lakes represent the more valuable fisheries, notably Derwentwater, Crummock and Hawes Water.

An increase in the abundance of fish is normally correlated with an increase in the abundance of plankton organisms, the phytoplankton forming the ultimate source of this increase. This correlation is so well established that we may regard the increase of fish in the more evolved lakes as being due to the physico-chemical conditions, which induce a more abundant phyto- and zoo-plankton in these lakes, and hence *the fundamental factor in the abundance of fish becomes in this case the stage of development shown by any particular lake.* Apart from the increase in the bulk of plankton, this factor operates in another way, for, as a lake develops, silts become richer and more abundant, and *rooted vegetation* more luxuriant. Hence there are increased areas on which epiphytic algæ can grow, and increased shelter and food for the smaller aquatic animals. The submerged vegetation of Esthwaite, as an extreme example, is literally teeming with life, and offers a marked contrast in this respect with vegetation from a primitive lake like Wastwater. Pond,* on different and theoretical grounds, also concludes that abundant rooted vegetation is favourable to fish production. He shows that rooted submerged vegetation draws its mineral nutriment chiefly from the substratum. Hence he argues that the seasonal decay of this vegetation enriches the water in salts at the expense of the soil, in this way favouring an increase in the bulk of the plankton. It must be remarked that Pond's assumption can only hold where organic decay is rapid and complete, and it is therefore more likely to operate in relatively evolved lakes where silts are abundant and rich than in waters where they are scarce, and the rate of organic decay is consequently slow. In the latter type of water, organic matter accumulates in an undecayed condition.

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* 'The Lake District Fisheries,' London, 1898.

Kofoid,† however, concludes that the bulk of plankton (and hence of fish) is *inversely* proportional to that of the larger submerged plants. In the lakes to which he refers, the larger plants are almost entirely *Ceratophyllum*, a free floating form which must draw the whole of its mineral nutriment from the lake waters, and hence directly compete with the plankton. It is clear, from Kofoid's account of Flag Lake, that he recognises that an abundant *rooted* vegetation probably, on decaying, favours the production of a rich plankton. Hence his views are consistent with those advanced above, since the larger free-floating plants are absent from this lake area.

It would thus appear that the increase in rooted vegetation, in the bulk of the plankton, and in the abundance of fish, are all changes to be correlated one with another, and ultimately depend upon the topographic development of the lake.

No satisfactory evidence is as yet available to account for the decrease in numbers of trout in the evolved lakes. The indications are that trout food (mollusca, flies, larvæ and fresh-water shrimps) is most abundant in evolved lakes. It can only be suggested that the decline of this fish may be due to

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* 'Rep. U.S. Comm. Fish.,' 1905, p. 485.

† 'Bull. Ill. State Lab. Nat. Hist.,' vol. 6, p. 95 (1899).

(i) the silting up of the stony feeding grounds or their covering with algæ (*e.g.*, *Cladophora*), as suggested by Weiss;* (ii) the development of summer "stagnation" and a deficiency of oxygen in the deeper waters of the evolved lakes. Both of these factors are apparently in operation, and both are dependent upon the other physical characters of the lake basins. Thus, though the intermediate links in the causal chain await further study, it is probably safe to assume that the changes in type and numbers of the fish in these lakes are intimately correlated with the physical development of the lake basins.

Summary.—The data presented show that a very close connection exists between the flora (and fishes) of the English Lakes and the physical and chemical conditions of their shores and waters. These lakes are of the same age (glacial), of similar origin, and lie among rocks possessing relatively uniform characters. It is therefore possible to attribute the differences they show to variations in the rates of erosion and sedimentation of the lake basins, due to inequalities in the durability of the underlying rocks. In distinguishing *rocky* from relatively *silted* lakes, a contrast is therefore made between *primitive* and more highly *evolved* lakes, and it becomes possible to describe the stages in the post-glacial development of a rock-basin. The conception has particular value biologically, since it permits the study of the post-glacial development of vegetation. The two extreme phases, here distinguished, differ in the following particulars:—

Extreme Lake Phases.

	Primitive.	Evolved.
Percentage of drainage system cultivable	5–8	17–45
„ lake shore rocky (to 9 m.)	73–47	27–12
„ *Isoëtes* and *Nitella*	85–74	52–30
„ *Juncus fluitans*	8–5	2–0
„ *Potamogeton* and *Naias*	½–2	30–55
Phytoplankton	Desmids dominant	Diatoms dominant
Fish	Chiefly trout	Chiefly perch and pike

Evidence is adduced for considering all these differences as ultimately dependent upon the physical condition of the lakes.

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* Weiss, F. E., "Preliminary Account of the Submerged Vegetation of Lake Windermere, etc.," 'Proc. Lit. and Phil. Soc., Manchester,' vol. 53 (1909).

APPENDIX I.—Dimensions, Altitudes, and Geological Sites of the English Lakes.

	Length in miles.	Mean breadth in miles.	Area (square miles).	Greatest depth (feet).	Percentage area over 50 feet.	Altitude (feet).	Rocks underlying	
							Lake.	Drainage area.
Wastwater	3·00	0·37	1·12	258	77	204	Borrowdale Little granite	Borrowdale
Ennerdale	2·40	0·46	1·12	148	47·4	369	Granite Skiddaw	Borrowdale Granite
Buttermere	1·26	0·34	0·36	93	62·2	331	Skiddaw	Skiddaw
Crummock	2·50	0·40	0·97	132	73	321	Skiddaw	Skiddaw Little granite
Derwentwater	2·87	0·73	2·06	72	7	238	Skiddaw	Borrowdale
Bassenthwaite	3·83	0·55	2·06	75	6	225	Skiddaw	Skiddaw
Hawes Water	2·33	0·23	0·54	190	36	694	Borrowdale	Borrowdale
Coniston	5·41	0·33	1·89	184	62·9	146	Silurian	Borrowdale
Windermere	10·50	0·55	5·69	219	·58	134	Silurian	Borrowdale
Ullswater	7·35	0·47	3·44	205	64·8	477	Borrowdale Skiddaw	Borrowdale
Esthwaite	1·50	0·33	0·51	70	—	217	Silurian	Silurian

APPENDIX II.—Water Analysis of English Lakes.

Figures—parts per million.

	Dissolved solids.			$Na_2O + K_2O$.	CaO.	MgO.	$\frac{\text{Alkalies}}{CaO + MgO}$.	$\frac{Fe_2O_3}{Al_2O_3}$.	CO_2.	SO_3.	Cl_2.	SiO_2.	NO_3.	Hardness.*	
	Total.	Organic.	Mineral.											Temporary.	Permanent.
1. Wastwater	42·8	4·3	38·5	13·1	2·4	1·8	3·1	tr.	3·9	10·1	5·7	1·4	—	—	1·7
2. Ennerdale	30·6	5·6	25·0	7·3	2·4	1·2	2·0	tr.	2·4	4·6	5·7	1·4	—	—	1·4
3. Buttermere	52·5	6·4	46·1	15·4	2·8	2·3	3·0	0·4	3·2	13·9	6·6	1·4	—	—	1·8
4. Crummock	66·5	8·0	58·5	15·2	4·0	2·6	2·4	1·3	2·4	24·4	7·1	1·4	—	—	1·8
5. Hawes Water	47·1	4·3	42·8	12·3	4·0	3·6	1·6	0·1	7·1	5·7	7·1	2·8	tr.	0·3	2·0
6. Derwentwater	49·9	2·4	47·5	7·7	9·5	1·5	0·7	0·7	3·1	11·4	11·9	2·1	tr.	—	2·1
7. Bassenthwaite	57·0	6·1	50·9	8·3	7·9	3·5	0·7	0·4	5·7	14·9	7·1	2·8	0·4	—	2·0
8. Coniston	49·9	7·1	42·8	11·2	4·8	4·3	1·2	0·3	8·5	2·2	8·6	2·8	tr.	0·3	2·0
9. Windermere	59·8	10·8	49·0	5·4	10·1	1·4	0·5	0·4	6·4	13·1	8·5	3·3	2·0	1·0	1·8
10. Ullswater	54·2	11·4	42·8	10·6	5·6	3·1	1·2	0·1	7·8	4·2	7·1	4·3	2·0	0·3	1·7
11. Esthwaite	75·9	13·8	62·1	10·4	9·5	5·1	0·7	0·7	7·6	18·7	8·6	3·0	2·3	0·3	2·8
Mean 1–4	—	6·1	—	12·7	2·9	2·0	2·6	—	3·0	—	—	1·4	—	—	1·7
„ 5–7	—	4·3	—	9·4	7·2	2·9	1·0	—	5·3	—	—	2·6	tr.	0·1	2·0
„ 8–11	—	10·8	—	9·4	7·6	3·5	0·9	—	7·6	—	—	3·3	1·6	1·5	2·1

* As calcium carbonate—parts per 100,000.

A Method for Investigating the Hæmolytic Activity of Chemical Substances.

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(Communicated by Sir E. Sharpey Schäfer, F.R.S. Received May 13, 1921.)

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Introduction.

Arrhenius has pointed out that the phenomenon of hæmolysis may be described by the qt. rule.* In this investigation it has been found that this rule is quite insufficient to describe the relation between the quantity of a hæmolytic agent and the time which such quantity takes to produce hæmolysis, unless a large margin of error be admitted. Since in some of the examples quoted by Arrhenius, the end point was not complete hæmolysis, but a certain percentage, it is easy to see that the difficulties of investigation account for the fact that the discrepancy between results given by experiment and those obtained by the use of the qt. rule has been overlooked. Further it is recognised that this rule is not of general application; being inapplicable, for instance, in the case of sodium oleate. In order to remove the difficulty of investigation, a technique of great accuracy has been introduced; the results of experiments will be found to require a description other than that furnished by the qt. rule.

Special Apparatus Required.

(1) A set of pipettes to deliver 1, 2, 3, 4 and 5 c.c.

(2) Pipettes, 1 c.c. capacity, graduated in 0·01 of a cubic centimetre.

(3) Burettes and pipettes of convenient capacity, and tared flasks of 50 and 100 c.c. capacity.

All volumetric apparatus must be certified, or preferably calibrated by the experimenter.

(4) Tubes, about 1 × 3 inches, with flat bottoms for preference; these have rubber stoppers, with one perforation for the passage of a thermometer.

(5) About twelve thermometers, graduated from 0° C. to 50° C. and capable of being read to 1/10 of a degree: these must all be checked against a standard instrument. The bulbs should be small, and the scale arranged so that it shall be above the level of the stopper of the tube when the thermometer is in position.

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* Arrhenius, S., 'Quantitative Laws in Biological Chemistry,' 1915, p. 63, *seq.*

(6) Three water baths, for maintaining temperatures at about 3° C., 12° C. and 30°—50° C. respectively. These should each be capable of containing about six of the tubes above mentioned in such a way that the degree of hæmolysis in the contents of the tubes may be seen without removing the tubes from the bath. The baths are arranged so that the contents of the tubes are seen against a screen lit by artificial light.

Preparation of the Standard Blood Suspension.

The suspension used in all the experiments is of an arbitrary, but convenient strength.

It is prepared as follows:—0·5 c.c. of blood is drawn from the finger; the blood must flow easily, and the finger must not be squeezed. This volume of blood is added to a tube containing 15 c.c. of 1·5 per cent. citrated saline; the receiving pipette should be rinsed with this saline, and drained, before drawing up the blood. The contents of the tube are then centrifuged, the clear supernatant fluid removed, and the tube again filled, this time with 0·95 per cent. sodium chloride. Again it is centrifuged. This process is repeated, so as to make four washings in all. After the final washing, the supernatant saline is removed, the tubes put in the centrifuge for a few minutes, and the last drops of saline taken off the cells with a capillary pipette. The cells are then added to 10 c.c. of 0·95 per cent. sodium chloride.

The resulting blood suspension will keep for fully eighteen hours, but should be used soon after preparation. In carrying out series of experiments, it is important that the blood used should be derived from the same person, *e.g.*, from the experimenter. During more than a year's work, involving the preparation of nearly a thousand such suspensions, and using the blood of the same person, a variation of the suspension sufficiently great to be detected has never occurred. It may, therefore, be taken that the suspension, if carefully prepared, is reliably constant in strength; it is, however, most necessary that the cells be freed from all traces of serum.

All the formulæ given refer to this suspension: the modifications of the formulæ required if suspensions of other strength are used will be given later.

Technique.

To investigate the hæmolytic activity of a hæmolytic substance, the following technique is employed. Since the activity of such a substance depends on (1) the dilution of the substance, (2) the quantity of blood suspension which it has to hæmolyse, (3) the time which it takes to complete this hæmolysis, and (4) the temperature at which the experiment is conducted, each of these variables must be controlled or measured: where a standard blood suspension is used, (2) may be neglected.

1. A solution of convenient strength in 0·95 per cent. NaCl of the hæmolytic substance to be investigated is prepared: as a rule, a solution 4 c.c. of which contains 50 mgrm. of the substance is employed. In the case of the bile salts, a solution of about this strength is essential; it is further very convenient to have the solution so adjusted, as it facilitates calculation of dilutions. From this solution dilutions are made as required. A series of dilutions are made, *e.g.*, 1·1000, 1·2000, 1·3000, 1·4000. 4 c.c. of each dilution is placed in a series of tubes with thermometers, as described above. The tubes are then placed in the water bath at, say, 40° C.; with them is placed a tube containing blood suspension. When the temperature of each tube has reached the temperature of the bath, as indicated by the thermometers carried by each, the blood suspension is added and the experiment commenced. This requires care and practice to perform satisfactorily and is done as follows: The tube containing the blood suspension is inverted rapidly, to mix the contents, and is then replaced in the bath. The pipette of 1 cc. capacity with which the measured quantity of blood suspension is to be added to each tube, is warmed by drawing up through it saline which has been warmed to a moderate heat over a flame. It is then at once dipped into the tube containing the blood suspension, of which 1 c.c. is drawn up; this is delivered without delay into the tube containing the strongest solution of hæmolytic agent (in the above series the 1·1000 tube). The time is noted; the process of adding blood suspension is repeated in the case of each of the tubes containing dilutions of hæmolytic agent, the moment of adding the blood being observed in each case. A stop-watch is almost essential. The operation requires to be carried out as quickly as possible. There should be scarcely an appreciable loss of temperature in the contents of the tubes when the blood is added. This result can be attained by speed and practice; although the method may appear clumsy it is open to fewer objections than is the method of adding the suspension by upsetting a small tube in which it is contained, and which is placed inside the large tube. It is possible to carry out this operation for ten tubes within thirty seconds, and with a loss of no more than 1/10° C. From the moment of adding the suspension the temperature in each tube should remain constant.

The moment of complete hæmolysis in the case of any tube is decided preferably by comparison with a fully hæmolysed control tube, which is placed in the bath. The water bath being placed against an illuminated screen, it is easy to compare the intensity of blackness with which a black rod placed horizontally across the screen is seen, in the case of tube and control respectively. This method of deciding the end-point is very satisfactory, as, if the tube be inspected by transmitted light alone, without a dark background, the time taken for complete hæmolysis will be much underestimated. The moment when complete hæmolysis occurs is noted; the time taken for the particular dilution of hæmolytic agent to hæmolyse 1 c.c. of standard blood suspension at the particular temperature employed is then known.

If the time for hæmolysis is long, the contents of the tubes should be stirred with the thermometer each half-hour.

If 4 c.c. of the dilution of hæmolytic agent contain 5 mgrm. (that is, a 1·10 dilution of the original solution prepared), then, when 1 c.c. of the blood suspension is added, the 5 c.c. in the tube will contain 5 mgrm. of hæmolytic agent, or a 1·1000 dilution (ignoring the negligible volume of the erythrocytes). In this way the dilution employed for producing hæmolysis is kept in round figures and readily calculable.

This method of investigating hæmolytic activity has been applied to a number of hæmolytic substances. Those investigated were the following:—

Saponin.
Sodium taurocholate.
Sodium glycocholate.
Lactic acid.
Acetic acid.
Citric acid.
Benzoic acid.
Ammonium chloride.

It has been found possible to describe the action of these substances by formulæ.

It is to be remembered that, while the general forms of these formulæ are correct for the action of these substances on blood suspensions of any concentration, the values of the various constants in the equations will vary for blood suspensions of different concentrations, and even for suspensions of the same concentration, of blood cells derived from different individuals or animals.

Relation between Time and Temperature.

In the cases of all hæmolytic substances examined, it has been found by experiment that there is a definite relation between the time taken for a given quantity of the substance to produce complete hæmolysis of 1 c.c. of standard blood suspension, and the temperature at which the experiment is conducted. As the temperature is increased, the time taken to produce complete hæmolysis becomes less.

If $T =$ the time, in minutes, taken to produce hæmolysis of 1 c.c. standard blood suspension,

and $\tau =$ the temperature at which the experiment is conducted, in degrees centigrade, the relation between T and τ is expressed by a hyperbola, of which one asymptote is the straight line $T = 0$. The relation is therefore described by the equation

$$\tau = \frac{\alpha}{\beta}[\beta - T] + \frac{\gamma}{T}.$$

where α, β, and γ are constants which depend on the quantity of hæmolytic substance whose action the hyperbola describes.

In order to find by experiment, within the limits of experimental error, the values of α, β, and γ for a given quantity of hæmolytic substance, the following procedure is used:—

(1) Find by experiment the time taken by this quantity of hæmolytic substance to produce hæmolysis of 1 c.c. of standard blood suspension, when τ_0 = any value near 0. Call this time T_0.

(2) In a similar manner find T_1, when τ_1 = any value near 10.

(3) Similarly find T_2, when τ_2 = any value between 30 and 45.

(4) Treat that part of the hyperbola which describes the relation between T and τ, when τ is any value from 0 to 10, as a straight line, which will pass through the points T_0 and T_1: determine the intercepts made by this line on the axes of T and τ respectively. The intercept on the T axis will equal β; that on the τ axis will equal α.

(5) Filling in the values of α and β, obtained as above (4), and the values of T_2 and τ_2 obtained experimentally in (3), in the equation

$$\tau_2 = \frac{\alpha}{\beta}(\beta - T_2) + \frac{\gamma}{T_2},$$

find the value of γ for the hyperbola describing the relation between T and τ, for the particular quantity of hæmolytic substance under investigation.

The equation expressing the relation between T and τ for any given quantity of hæmolytic substance may thus be found from three experiments. The values of α, β and γ obtained should be checked by comparing experimental and calculated values of T, when $\tau = 20$, $\tau = 30$, $\tau = 40$, and $\tau = 50$. If α and β are accurately determined, an excellent correspondence between calculated and experimental results will be obtained.

In investigating the hæmolytic action of a substance, the equations for hyperbolas representing the relation between T and τ, in the case of each of several dilutions of the substance, are obtained. A series of values of α, β, and γ are thus found, corresponding to various dilutions: these constants may then be expressed as functions of the dilution.

General Equations relating α, β, and γ to the Dilution.

In the cases of all the hæmolytic substances examined, these constants are related to the dilution in a similar way :—

(1) The constant α is a linear function of the dilution. If δ be the number of cubic centimetres which contains 1 grm. of the hæmolytic agent, in the dilution investigated, then the relation between α and δ is expressed by an equation of the form

$$\alpha = m\delta + n,$$

where m and n are constant for the particular hæmolytic substance.

(2) The constant β is related to δ by a curve. If on the curve points, p_1, p_2, p_3, etc., corresponding to values, δ_1, δ_2, δ_3, etc., be joined to the origin $\beta = 0$, $\delta = 0$, a series of angles θ_1, θ_2, θ_3, etc., will be formed between the joining lines and the abscissa. These angles, expressed in degrees, are related to δ_1, δ_2, δ_3, etc., by a hyperbola : the relation being expressed by the equation

$$0{\cdot}01\delta = \frac{a}{b}[b-\theta] + \frac{c}{\theta},$$

since the hyperbola has one asymptote, the straight line $\theta = 0$. The relation between β and δ is therefore expressed by the equations

$$0{\cdot}01\delta = a - \frac{a\theta}{b} + \frac{c}{\theta}, \qquad \text{(i)}$$

$$0{\cdot}01\delta = \beta \tan\theta, \qquad \text{(ii)}$$

where a, b and c are constant for the particular hæmolytic substance.

(3) The relation between γ and δ is expressed by an equation of the form

$$\gamma = p\delta,$$

where p is a constant for the particular hæmolytic substance. Since α, β, and γ are related to the dilution in these ways, by a knowledge of the equations given above, and of the values of a, b, c, m, n, and p, for a hæmolytic substance, it is possible to calculate the time taken by any dilution of that substance to produce complete hæmolysis of 1 c.c. of standard blood suspension, at any given temperature, or to make other calculations involving these variables.

In order to illustrate these general relations, the results obtained from an examination of the hæmolytic action of three substances will be given in detail. The substances selected—saponin, sodium taurocholate, and lactic acid—are chosen because they are examples of a highly hæmolytic agent, a moderately hæmolytic agent, and a feebly hæmolytic agent respectively :—

1. *Saponin.*

The following values were obtained for the constants in the equations relating to T and τ:—

δ.	α.	β.	γ.
5000	9·4	25	4·1
10000	10·4	57	8·3
20000	12·4	139	16·6
30000	14·4	252	25

From these, the following values are calculated for the constants in the equation relating α, β and γ to δ:—

a.	*b.*	*c.*	*m.*	*n.*	*p.*
1300	65	0·4	0·0002	8·4	0·00083

The correspondence between observed and calculated results is shown by the following Tables:—

$\delta = 5{,}000.$

τ.	$T_{\text{exper.}}$	$T_{\text{calc.}}$
10	2·3	2·5
30	0·17	0·2

$\delta = 20{,}000.$

τ.	$T_{\text{exper.}}$	$T_{\text{calc.}}$
12	16	15
40	0·4	0·5

$\delta = 50{,}000.$

τ.	$T_{\text{exper.}}$	$T_{\text{calc.}}$
40	2·3	2

2. *Sodium Taurocholate.*

Certain points have to be noted regarding this salt.

(1) A pure dry specimen must be obtained. Dryness is ensured by desiccating *in vacuo* for about a fortnight.

(2) Sodium taurocholate does not form a true solution. In saline, water, or dilute alcohol, it becomes opalescent, and when in this state will all pass through a fine filter. Its behaviour is similar to that of a soap. The rapidity with which this opalescence forms depends on the dilution, a 1 per cent. solution remaining clear for some time, while a 0·1 per cent. solution becomes rapidly cloudy. The stability is lessened by heating. Consequently it is necessary (*a*) to make the original solution, from which dilutions are to be made, not more dilute than 1 per cent.; (*b*) to make the dilutions and perform the experiments as rapidly as possible; and (*c*) to avoid heating the solution unnecessarily.

The following values were obtained for the constants in the equations relating T and τ:—

δ.	a.	β.	γ.
1000	15	9	20
2000	19	31	40
2500	21	57	50
3000	23	100	60
3500	25	200	70
4000	27	570	80

From these are derived the following values for the constants in the equations relating α, β, and γ to δ:—

a.	b.	c.	m.	n.	p.
40	65	10	0·004	11	0·02

The correspondence between calculated and experimental results is shown by the following tables:—

$\delta = 1000$.

τ.	$T_{\text{exper.}}$	$T_{\text{calc.}}$
10	5	5·3
30	1	1·2

$\delta = 2000$.

τ.	$T_{\text{exper.}}$	$T_{\text{calc.}}$
20	7	7·3
30	3	3

$\delta = 3000$.

τ.	$T_{\text{exper.}}$	$T_{\text{calc.}}$
10	60	60·7
40	3·5	3·4

$\delta = 4000$.

τ.	$T_{\text{exper.}}$	$T_{\text{calc.}}$
12	330	324
40	6	6·3

$\delta = 7000$.

τ.	$T_{\text{exper.}}$	$T_{\text{calc.}}$
45	22	23·1

3. *Lactic Acid.*

This is a feebly hæmolytic agent. The end point is difficult to determine, especially at high temperatures, owing to colour changes produced in the hæmoglobin. The following values were obtained for the constants in the equations relating T and τ:—

δ.	a.	β.	γ.
1000	26·6	64	40
1250	29·1	152	50
1666	33·2	389	66
1785	34·4	477	71
2000	36·6	636	80

From these are derived the following values for the constants in the equations relating α, β and γ to δ:—

a.	b.	c.	m.	n.	p.
9	65	20	0·01	16·6	0·04

The following tables compare calculated and experimental results:—

$\delta = 1000$.			$\delta = 2000$.		
τ.	$T_{exper.}$	$T_{calc.}$	τ.	$T_{exper.}$	$T_{calc.}$
10	42	42·3	30	125	127
30	10·5	10·6	40	47	48

The general relations between T, τ and δ, for any of the hæmolytic substances examined, having been dealt with, there remains to be expressed certain relations which exist between the constants of the equations relating α, β and γ to the dilution. These relations hold true for all the substances examined, and probably for most hæmolytic substances.

It will be seen that the hæmolytic activity of a substance depends principally on the manner in which α and β vary with the dilution. The variation of α with the dilution depends chiefly on the value of m, while the variation of β with dilution depends chiefly on the value of a. There must, then, be a relation between the value of n and the value of a, for any substance.

Let $$a/b = \tan\phi,$$

and $$100m = \tan\omega,$$

the relations between ϕ and ω is expressed by a hyperbola, whose equation is

$$0{\cdot}75\,\omega^2 + \omega(\phi - 45) - 52 = 0.$$

Since b is a constant for all hæmolytic substances examined (65), from a known value of a the corresponding value of m can be found.

The relation between α and δ further depends on the value of the constant n. This constant is related to the value of m. If, as above,

$$100m = \tan\omega,$$

the relation between ω and n is expressed by a rectangular hyperbola, and by the equation,

$$n(90 - \omega) = 750.$$

The relation of β to the dilution depends to some extent on the value of c; this constant has a simple relation to the constant p,

$$c = 500p.$$

The value of c may be readily found for any substance, for which one value of γ is known.

From a knowledge of these general relations, if three suitable experimental readings be given for a substance, the time taken by it to produce hæmolysis under any circumstances of temperature or dilution may be calculated.

As an example:—Using acetic acid (S.G. = 1·044), it is found that the following Table gives the relation between certain values of T and τ, when $\delta = 1000$,

τ.	T.
2	135
7	100
44·1	4

From these values may be found,

$$\alpha = 36{\cdot}4$$
$$\beta = 143$$
$$\gamma = 40$$

From the value of γ we find first p and then c. From the values of β and δ the value of θ in the equation relating δ and β may be found. Since c is known, the value of a in this equation may be calculated, b being taken as 65. When a and b are known, ϕ may be calculated and from it the value of ω. This value gives the values of m and n. Thus, we may find for acetic acid that the following values are true:—

a.	b.	c.	m.	n.	p.
5	65	20	0·0146	21·8	0·04

The constants being known, the values of α, β, and γ for any dilution may be calculated. For instance, when $\delta = 1785$,

α.	β.	γ.
37·8	1136	71

The value of T corresponding to any value of τ for this dilution may be found in the usual way; for example,

$$\delta = 1785.$$

τ.	$T_{\text{exper.}}$	$T_{\text{calc.}}$
32	185	188·7

While it is thus possible to calculate with considerable correctness the relation of T and τ for any dilution of a substance for which only three experimental readings are given for one dilution, it is advisable to take three readings for each of several dilutions, the accuracy of the determination of the constants a, b, c, m, n, and p being greatly increased thereby. If a sufficient number of experimental readings for various dilutions are available, results may be obtained graphically.

Summary.

1. A technique for the investigation of the hæmolytic action of chemical substances is described.

2. The relation between the time taken by a given quantity of hæmolytic substance, and the temperature at which it acts, is expressed by a hyperbola.

3. Equations are given expressing the relation between the constants of such a hyperbola and the quantity of hæmolytic substance to which the hyperbola applies.

4. Certain general relations, which have been found to hold for all substances examined in connection with this research, are pointed out.

5. A comparison between experimental and calculated results is given.

Observations on the Effects of Fat Excess on the Growth and Metamorphosis of Tadpoles.

By Robert McCarrison, M.D., D.Sc., LL.D., Lt.-Colonel I.M.S.

(Communicated by Prof. C. S. Sherrington, Pres.R.S. Received June 10, 1921.)

(From the Physiological Department, University of Oxford.)

Previous experimentation* has shown that in certain circumstances the presence of excessive amounts of fat in the food of animals may be harmful. Thus, an excess of butter in association with a dietary of autoclaved rice hastens the death of both pigeons and monkeys, and gives rise to changes in the internal organs more pronounced than those resulting from an autoclaved rice dietary alone. Again, an excess of butter in association with a dietary of mixed grains and peas causes enlargement, with hyperplasia and vesicular budding, of the thyroid gland in pigeons, identical with that characteristic of Graves' disease.† This enlargement of the thyroid gland is associated with a reduction in size of the adrenal glands. If, however, fresh onions be added to the dietary of mixed grains and butter both the incidence of the thyroid enlargement and the intensity of the hyperplasia are reduced; while the associated diminution in size of the adrenal glands is not so marked.

This observation as to the effect of an otherwise adequate food containing an excess of butter in producing thyroid hyperplasia of the Graves' disease type has received confirmation from the work of E. and M. Mellanby, who have recently* reported identical thyroid changes in puppies to whose food butter had been added. Mellanby has further recorded that not only does butter, and in a lesser degree certain other fats, bring about these changes but they do not arise when the fat used is cod-liver oil.

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* McCarrison, R., 'Ind. Jour. Med. Res.,' vol. 6 (4), p. 550, and vol. 7 (2), p. 308 (1919).

† McCarrison, R., 'Ind. Jour. Med. Res.,' vol. 7 (3), p. 633 (1920).

The innocuous action of cod-liver oil may possibly have some relation to the iodine-content of this oil, which in crude specimens may reach a concentration of 1 in 2,000. It was, therefore, thought desirable to examine, by experimental methods, what the relationship between fat metabolism and iodine intake might be, and the relationship of thyroidal activity to both.

The present communication is a preliminary account of the observations so far made. It is yet too early to provide an explanation of them, but their record will serve to direct attention to etiological and nutritional problems concerned with what may be called the "fat-iodine-thyroid balance."

Tadpoles were selected as suitable for the purposes in view by reason of the fact that their rate of growth and of metamorphosis is so sensitive to influence by iodine and thyroid extract. It was thought that this sensitiveness might serve as an index of the changes that are induced in the thyroid gland by an excess of fats.

Details of Experiments.

(1) Butter, lard, cocoa-nut oil, oleic acid, linseed oil, cod-liver oil, and arachis oil were chosen as the fats to be tested; these have a wide range of iodine values. The cod-liver oil was of the crude variety—the so-called "cattle cod-liver oil"—its iodine-content was estimated by Mr. A. A. F. Peel and found to be 0·002 per cent. He also found that the linseed oil contained no iodine.

(2) The tadpoles were hatched from the same batch of eggs. They were selected so as to be of approximately the same size. The experiments commenced on the eleventh day of their life.

(3) Fifty tadpoles were placed in each of the twenty-four dishes required for the experiments. There was one litre of tap-water in each dish. The dishes were arranged in three series of eight each. The water was changed daily, and the dishes thoroughly cleansed.

(4) A basal diet was provided consisting of flour, eighty-five parts, and caseinogen, fifteen parts. Fresh pond weed was supplied: during the first six weeks it was changed every third day, thereafter daily. This diet contained an adequate supply of proximate principles, of vitamins and of vegetable matter. The tadpoles ate it with avidity and their rate of growth was vigorous. They were fed daily.

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* Mellanby, E. and M., 'Proc. Physiol. Soc.,' vols. 7 and 10 (March 12, 1921).

(5) The fats to be tested were added (either alone or after admixture with known quantities of iodine) to the basal diet in the proportion of approximately 1 grm. of the fat to 1 grm. of the flour-caseinogen mixture. A dough of soft consistency was prepared therefrom, and the mass divided into a number of small pills which were supplied to the tadpoles.

(6) In the first series of eight dishes the effects of the various fats, as compared with controls receiving the same basal diet but without fats, were observed (fig. 1).

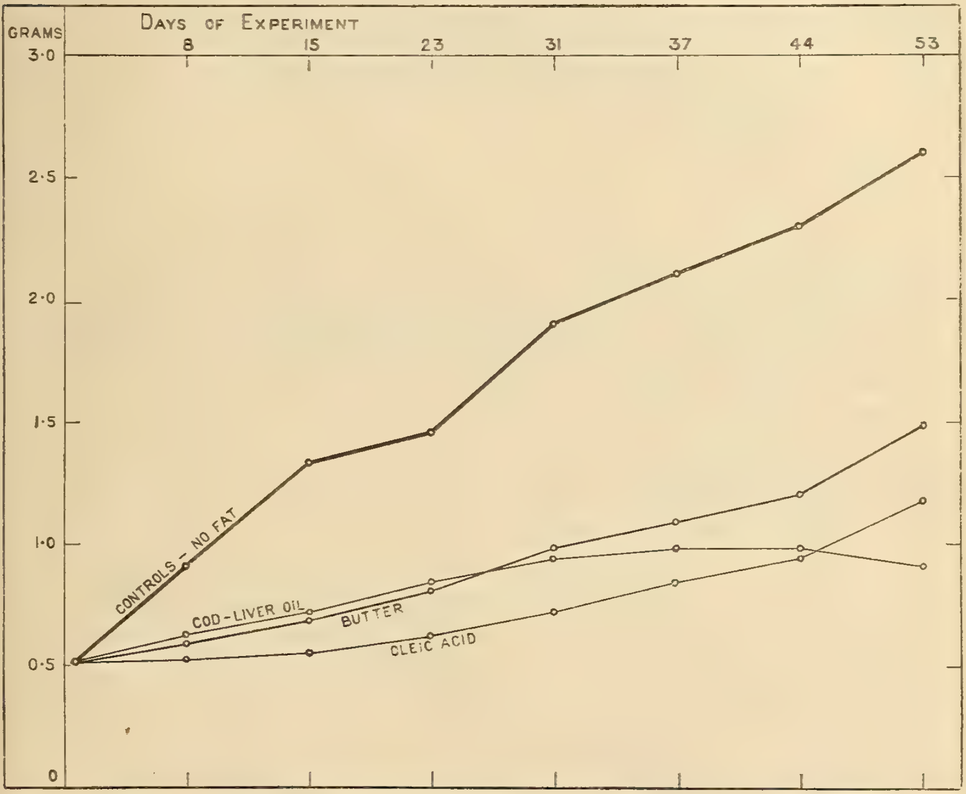

Fig. 1.—Showing the effects on growth of tadpoles of an excess of *oleic acid*, of *butter*, and of *cod-liver oil* in the food.

(7) In the second series of eight dishes the effects of the various fats after admixture with iodine, in the proportion of 0·5 mgrm. per gramme of food-mixture, were observed in contrast with controls receiving the same amount of iodine in the basal diet but without fats (figs. 2, 3, 4).

(8) In the third series of eight dishes the effects of the various fats after admixture with iodine, in the proportion of 1 mgrm. per gramme of food-

mixture, were observed in contrast with controls receiving the same amount of iodine in the basal diet but without fats (figs. 2, 3, 4).

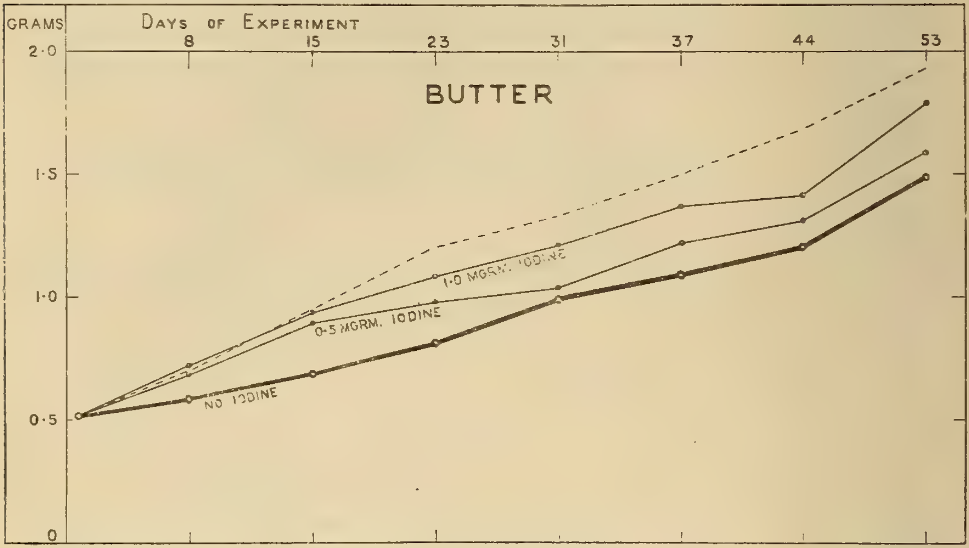

Fig. 2.—Showing the effects of 0·5 mgrm., and of 1·0 mgrm. of iodine in counteracting the retardation of growth induced in tadpoles by an excess of *butter* in their food. Dotted line shows the normal rate of growth of tadpoles receiving 1 mgrm. of iodine per gramme of basal diet, but *without butter.*

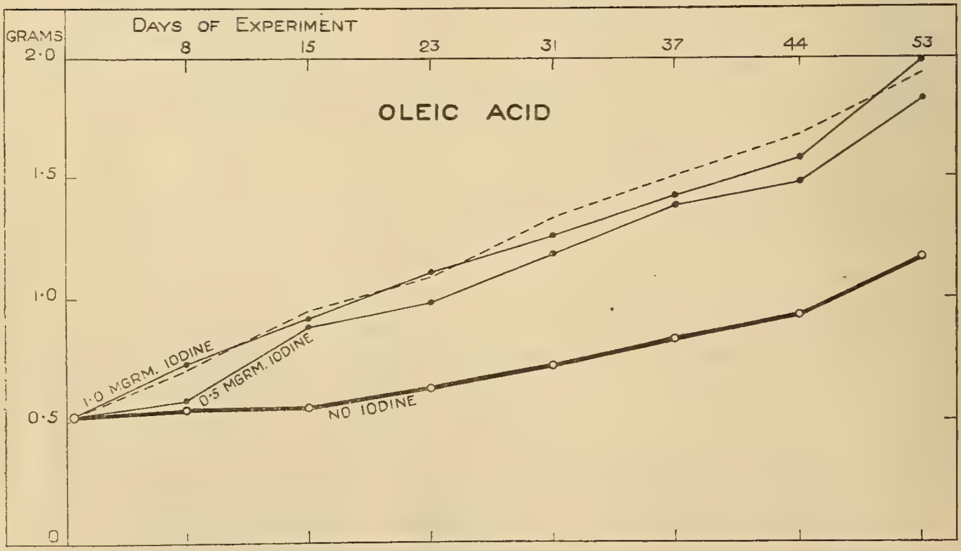

Fig. 3.—Showing effects of 0·5 mgrm. and of 1 mgrm. of iodine in counteracting the retardation of growth induced in tadpoles by an excess of *oleic acid* in their food. Dotted line shows the normal rate of growth of tadpoles receiving 1 mgrm. of iodine per gramme of basal diet, but *without oleic acid.*

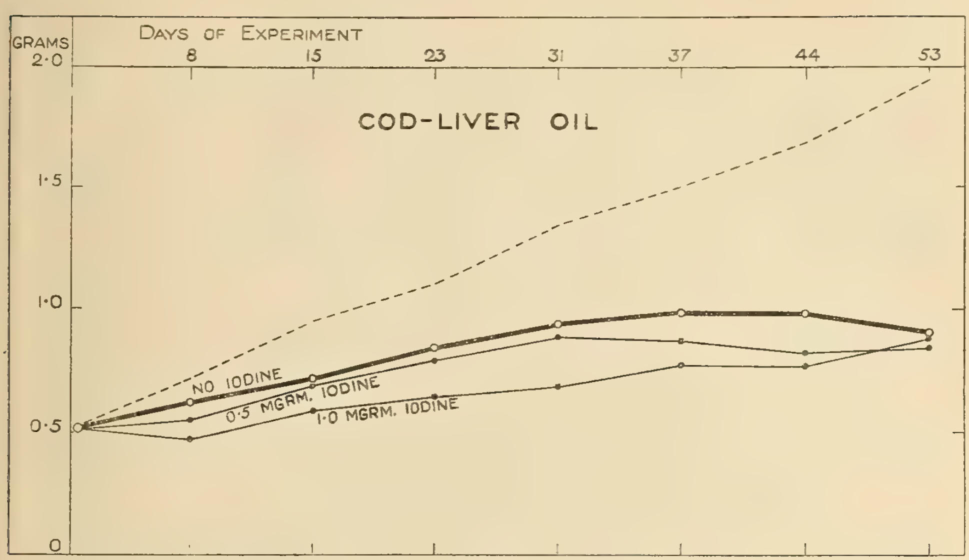

FIG. 4.—Showing the effects of 0·5 mgrm. and of 1 mgrm. of iodine in accentuating the retardation of growth induced in tadpoles by an excess of *cod-liver oil* in their food. Dotted line shows the normal rate of growth of tadpoles receiving 1 mgrm. of iodine per gramme of basal diet, but without *cod-liver oil.*

(9) The iodine was added in solution of which the following is the composition: iodine, 0·477 grm.; potassium iodide, 0·682 grm. (equivalent to 0·521 of iodine); water, 100 c.c. The iodine solution was intimately mixed with the fat in each case, and the flour-caseinogen mixture subsequently added to form the soft pills.

(10) Food intake: The tadpoles ate the food-mixtures with avidity during the earlier part of the experiment, and during this period the rates of growth varied in a conspicuous way (fig. 1). Later, however, certain differences in the food intake in different dishes were observed. Tadpoles receiving the basal diet without admixture with fats, ate well throughout the whole course of the experiment, so also did those receiving food-mixtures containing arachis oil. Those receiving cod-liver oil and linseed oil ate greedily at first and later much more sparingly; while those receiving the harder oils, butter, cocoa-nut oil and lard ate moderately, as did those receiving oleic acid mixtures. It is concluded, therefore, that while the wide variations in the rate of growth (fig. 1) between tadpoles receiving no fat and those receiving fat may, in some measure, have been due to a lesser food intake by the latter during the later stages of the experiment, the great retardation of growth induced by the fat was not due in the main to this cause.

(11) Temperature conditions—which have been shown by Julian Huxley

to modify greatly the rate of growth of tadpoles—were, as far as possible, uniform. In the case of the lard and arachis oil dishes, the animals had the advantage of more heat from the sun, as these dishes were placed on a bench near a sunny window. In general, however, the factor of variations in external temperature was not responsible to any appreciable degree for variations in the rates of growth or metamorphosis. The room temperature during the course of the experiments ranged between 65° and 70° F.

(12) The mortality was negligible during the first three weeks of the experiment; later, and especially after the fortieth day, it was considerable amongst tadpoles receiving fluid fats, much less so amongst those receiving the solid fats. The presence of iodine in the food-mixtures tended to reduce the death rate in tadpoles receiving fluid fats. Œdema was a frequent cause of death in certain dishes: controls, cod-liver oil, arachis oil and lard. It appeared to have no relation to the presence or absence of iodine. Cannibalism, so usual among tadpoles, was conspicuous by its almost total absence, a circumstance which demonstrates the complete nature of the food provided.

(13) Pigmentation varied greatly in different dishes: the most pigmented were in general the smaller individuals and those receiving the fluid fats; the least pigmented were in general the larger individuals and those receiving the solid fats. Tadpoles receiving butter were, on the whole, less pigmented than those receiving other fats, although exceptions to this generalisation occurred among tadpoles receiving food-mixtures containing the more solid fats. It seems not improbable that the various fats may have had different effects on the pineal body.

(14) Individual variation in size was a very notable feature in the case of tadpoles receiving oleic acid, whether with or without iodine. It was a noticeable feature also in controls receiving no fats, and in those receiving food-mixtures containing lard, cocoa-nut oil and butter. Little or no variation in size was found among tadpoles receiving food-mixtures containing arachis oil, linseed oil and cod-liver oil.

(15) The method of recording rates of growth was as follows: The tadpoles were weighed weekly. At the first two weekly weighings only twenty from each dish were weighed. Subsequently—and as variations in the rate of growth of different individuals became more obvious—all were weighed. They were removed from their dishes into a gauze net. The wet mass of tadpoles at the bottom of the net was then freed of adherent water by means of absorbent paper, and the mass turned into a known weight of water. The total weight was thus arrived at, and the weight of twenty was calculated therefrom for purposes of charting. This figure was selected so as

to avoid errors such as might arise in consequence of reduction in the original numbers by a high mortality in any particular dish.

(16) Finally, an experiment was devised to determine the effect of fats in hastening or retarding the abnormally rapid rate of metamorphosis induced by a large intake of iodine. In this experiment twenty tadpoles were used for each fat to be tested. The diet consisted of a modification of that originally used by Swingle, who produced metamorphosis very rapidly by means of a mixture of one part of metallic iodine to 100 parts of flour. In the present experiment the same proportion of iodine was employed, but with this difference, that instead of flour, a mixture of flour and caseinogen, in the proportion of eighty-five parts of the former to fifteen parts of the latter, was used. The fats to be tested were added in quantities sufficient to make a soft dough. Pond weed was not given to any of the tadpoles in this series. The fats tested were butter, cocoa-nut oil, oleic acid, linseed oil and cod-liver oil. The observations were controlled by forty tadpoles, twenty of which received the basal diet without fat or iodine, and the remaining twenty the basal diet with iodine but without fat.

Results of the Experiments.

A. *Observations on Growth:—*

(1) The rate of growth of tadpoles receiving an excess of the fats, in an otherwise adequate dietary, was greatly delayed (fig. 1). This retardation of growth was not attributable solely to a lesser food intake, although this may have been responsible in some measure for it, but was dependent on other and unknown causes. The fats tested ranged themselves in the following order with respect to the severity of the retardation of growth induced by them during a period of fifty-three days: arachis oil, cod-liver oil, linseed oil, cocoa-nut oil, oleic acid, lard and butter.

(2) Iodine in the proportion of 0·5 to 1·0 mgrm. per gramme of food-mixture compensated, in greater or lesser degree, for the retardation of growth induced by certain fats, namely, butter, oleic acid, cocoa-nut oil, lard and arachis oil (figs. 2 and 3). On the other hand, no favourable influence on the rate of growth was exercised by this dosage of iodine in the presence of linseed oil and cod-liver oil; the rate of growth was, on the contrary, further retarded (fig. 4). The effect of iodine in compensating for the retarded growth induced by fats was due either to its action in increasing the intake of food or to its action in improving food-assimilation, or to both. With respect to this action of iodine, the fats used group themselves into two categories: those in whose presence food-assimilation was favoured by

iodine; and those in whose presence food-assimilation was not favoured by iodine. The first category is further divisible into two sub-classes, according to the degree of the favouring influence of iodine on assimilation: those in which the retarded rate of growth induced by the fat was wholly or almost wholly compensated for by 1·0 mgrm. of iodine per gramme of food-mixture; and those in which the retarded rate of growth was favourably influenced, but not compensated for, by this dosage of iodine. In the first sub-class are included butter, lard and oleic acid (figs. 2 and 3); in the second, cocoa-nut oil and arachis oil.

B. *Observations on Metamorphosis.*

(1) Among tadpoles which received metallic iodine in the proportion of one part of iodine to 100 parts of food-mixture, but no pond weed, the following results were observed at the end of thirty days of experiment:—

(*a*) Tadpoles whose food-mixture contained butter showed well-developed hind limbs in every case; the stage of metamorphosis was in advance of controls which received the same amount of iodine but no fats, and was far in advance of those which received other fats. Butter thus hastened the abnormal metamorphosis induced by a large iodine intake.

(*b*) A similar but less uniform result was observed with regard to cocoa-nut oil. This oil hastened in certain individuals the normal metamorphosis induced by a large iodine intake.

(*c*) Oleic acid and linseed oil exercised little or no influence on the abnormally rapid metamorphosis induced by a high iodine intake.

(*d*) Cod-liver oil, on the other hand, markedly delayed the abnormally rapid metamorphosis induced by a high iodine intake, and at the same time the loss of weight induced by the iodine was less pronounced than in the case of other fats, oleic acid excepted.

It was observed that the hind limbs developing in the course of this experiment were unhealthy looking and sometimes shrivelled, especially in the case of linseed oil.

(2) Among tadpoles which received 0·5 to 1 mgrm. of iodine per gram of food-mixture and pond weed, the rate of metamorphosis was little affected in the absence of fats; the larger dose of iodine tended to hasten metamorphosis slightly. When, however, fats were present, it was observed that metamorphosis was markedly delayed by the fluid fats, and to a much less extent by the harder fats; and, that while iodine tended to compensate for the delay in metamorphosis induced by oleic acid and arachis oil, no such

tendency to compensation occurred in the presence of linseed oil and cod-liver oil.

Summary of Results.

(1) An excess in the food of tadpoles of the several fats used caused great retardation in the rate of growth.

(2) Iodine in amounts of 0·5 to 1·0 mgrm. of food-mixture tended to compensate for the retardation of growth induced by butter, lard, oleic acid, cocoa-nut oil, and arachis oil, but not for that induced by linseed oil and cod-liver oil.

(3) The normal rate of metamorphosis was but slightly affected by the harder fats: butter, cocoa-nut oil, and lard; but was delayed by the fluid and less saturated fats: oleic acid, arachis oil, linseed oil, and cod-liver oil.

(4) The delayed rate of normal metamorphosis induced by the fluid fats tended to be compensated for by the presence of small quantities of iodine in the food in the case of oleic acid and arachis oil, but was not compensated for by the same quantities of iodine in the case of linseed oil and cod-liver oil.

(5) The abnormal metamorphosis induced by a high iodine intake was considerably hastened by a high proportion of butter in the food-mixture, and to a lesser degree by a high proportion of cocoa-nut oil in the food-mixture, but was markedly retarded by a similar proportion of cod-liver oil in the food-mixture.

Conclusion.—It seems probable from these results that, in so far as certain fats—butter, lard, oleic acid, cocoa-nut oil, and arachis oil—are concerned, an iodine intake, proportionate to their intake in the food, is requisite for the maintenance of normal metabolism. The influence of cod-liver oil and of linseed oil in further retarding growth in the presence of an amount of iodine, that is favourable to growth in the case of other fats, is as yet not understood.

The histological findings in connection with the thyroid gland will form the subject of a further communication.

Experimental Researches on Vegetable Assimilation and Respiration. XIV.—*Assimilation by Submerged Plants in Dilute Solutions of Bicarbonates and of Acids: an Improved Bubble-Counting Technique.*

By A. J. Wilmott.

(Communicated by Dr. F. F. Blackman, F.R.S. Received June 28, 1921.)

The procedure of counting the bubbles given off from the cut stem of a submerged water plant, to obtain a measure of the magnitude of the plant's assimilation, was introduced by Dutrochet in 1837 and matured by Sachs in 1864. It has been of great use in demonstrations and class work, and has also been seriously employed in a number of researches. From time to time it has been subjected to a good deal of criticism, as giving a faulty measure of the true rate of photosynthesis. It is, however, a striking fact that nothing has been done to improve the technique of the procedure since the method was originally introduced.

The first part of the present paper describes a simple device which removes at once two of the very serious defects of the method, and renders it much more suitable for research work. The second part applies this method to an elucidation of the extraordinary effect of dilute mineral acids upon bubble rate brought forward by Treboux. In the third part the relation of bubbling in bicarbonates to bubbling in carbonic acid is investigated, and it is shown that Angelstein's statement that water plants can actively split bicarbonates in solution is erroneous.

Part I.—THE BUBBLE-COUNTING METHOD OF MEASURING CARBON-ASSIMILATION IN SUBMERGED PLANTS.

During the course of the investigations described in the later parts of this paper, which were carried out in the Botany School, Cambridge, in 1911, at the suggestion of Dr. F. F. Blackman, the bubble-counting method was carefully examined, and improved in several respects.

Various defects of this method had been pointed out before the present work, and, since that date, Kniep, in 1915, has destructively criticised it None of its critics seems, however, to have attempted to improve it. Yet the method is so extraordinarily sensitive to influences of all sorts that it cannot be lightly abandoned as a research instrument. As will be shown immediately, some of its most serious defects are quite easily remedied in simple ways. These may be considered under two headings: (A) Methods of avoiding errors in bubble-counting due to variations in size of the gas bubbles, and (B) Method of diminishing errors due to gaseous diffusion in the water round the plant.

(A) *Errors due to Variation in Size of Bubbles can be Avoided by the Use of Glass "Bubblers" and Bubbling Cup.*

Aa. *Variations in Size of Bubbles due to Alterations of Cut Stem Aperture.*—It is obvious that if, over a period of time, the size of the bubbles given off from the cut stem of a submerged water plant varies, either irregularly or by steady drift, then merely counting the number of bubbles gives no adequate measure of the volume of gas set free by the photosynthetic process. The papers of all workers who have used the bubble-counting method contain lamentations about the difficulty that this spontaneous variation produces in attempting long comparative experiments, yet no proposal has been brought forward for really overcoming it. Palliatives, such as cutting the surface overnight, pinching, scratching, covering with varnish and pricking, and so on, have been employed, but it is generally admitted that many experiments suffer from such obvious variations of bubble-size that they must be abandoned.

In the course of the experiments now described, it was found practicable to manufacture small glass nozzles to fit over the cut end of the shoot, and provide a fine glass capillary opening of absolutely constant size. These "bubblers," as they will be called, were made by first drawing out glass tubing to an external diameter of 1·5 to 2 mm., with an internal bore just sufficient to fit closely over the shoots to be experimented with. A short region of this tube was then sharply drawn out at right angles to a very fine capillary and broken off, to leave a minute aperture. Fitting such nozzles on to the cut stem ensures a long steady succession of bubbles all of the same size.

Ab. *Variations in Size of Bubbles with Alteration of Internal Gas-Pressure.*—Such changes are well known when the raw end of the plant is used. The openings of the plant capillary spaces are so fine that greatly increased rate of bubbling delivers smaller bubbles than does a slow flow of gas, and thus quite invalidates the counted numbers of bubbles. This source of error is also eliminated by the glass bubblers, because the apertures are larger than in such plants as Elodea, and the bubbles delivered are larger and come off much more slowly, so that a rate of flow which would affect their individual size is never reached in normal photosynthesis.

A nozzle that gives a bubble every 5 to 10 seconds is the most satisfactory size. It is found that, in constant conditions, the variation of timing five bubbles may not exceed 2 per cent. for several hours. Bubblers delivering even as slowly as one bubble in 8·5 minutes gave constant results to within 2 seconds.

One very great advantage of these bubblers is that the same one may be used for different plants in succession, and so strict comparisons made independently of the size of the plant's own capillary openings.

Unless the bubblers are quite clean, the bubbles may tend to cling to the tip. This can be cured by boiling them in a test-tube with distilled water.

With the comparatively large slow bubbles of the glass bubbler, it is easy to see that the range of natural rates does not affect the size of the bubbles. One never gets an almost continuous stream of very tiny bubbles, but, after each escapes, the water springs back a little way into the capillary nozzle and remains stationary for a bit while the internal pressure gets up; then it is quickly pushed out to give a nearly full-sized bubble, after which the bubble slowly increases to full size against the surface tension till the critical point is reached and it breaks away. This pulsating rhythm is repeated with great steadiness.

Ac. *Variations of Size of Bubbles due to Varying Surface-Tension of the Solution.*—A line of research, for which the bubble method has been used by Treboux, Jacobi, Pantanelli and others, is the study of the effect, on the rate of photosynthesis, of substances added to the water so as to penetrate into the plant. Any alteration of surface-tension of the water by these solutes must directly affect the size of the bubbles given off, so that bubble-counting ceases to give an accurate measure of the change produced in the assimilating cells. This complication can be completely avoided by the use of another simple device. The bubbles are in our experiments delivered not into the body of the experimental liquid, but into a small cup which contains distilled water, so that the tension is unaffected when solutions are changed. This cup is shown, together with the bubbler, in fig. 1. It consists of a glass tube 18 mm. wide, closed below by a cork, which has a hole through which the inverted plant stem is pushed, and secured above and below with a tenacious wax mixture of low melting point. The shoot is first attached to the cork and bubbler, and then the outer tube is slipped down on to the cork to form the bubbling cup.

Ad. *Variations of Size of Bubbles due to Osmotic Action of Liquids on the Stem Aperture.*—Pantanelli, Treboux and others have set out to investigate the effect of quite strong solutions of various salts upon photosynthesis in water plants. It is clear that, without special precautions, such solutions must shrink the cells bounding the stem aperture, and so enlarge the aperture, and probably the size of the bubbles.

That this effect really exists was demonstrated by some special experiments in which air was forced slowly by pressure through a short length of Elodea stem (one or two internodes), so that it escaped in bubbles at the free end. The other end was connected by rubber and glass tubing to a head of water forcing air through the plant. A scale was placed behind one of the intervening tubes, and the travel of the air-water meniscus at the head of the column of water was read for a given number of bubbles. When the index column of water had reached the end of the scale, the connection was interrupted and the index sucked back, so that further readings could be taken after re-connecting. The whole was so arranged that the final glass tube carrying the Elodea shoot, came up through a cork in the bottom of a vessel into which various liquids could be introduced.

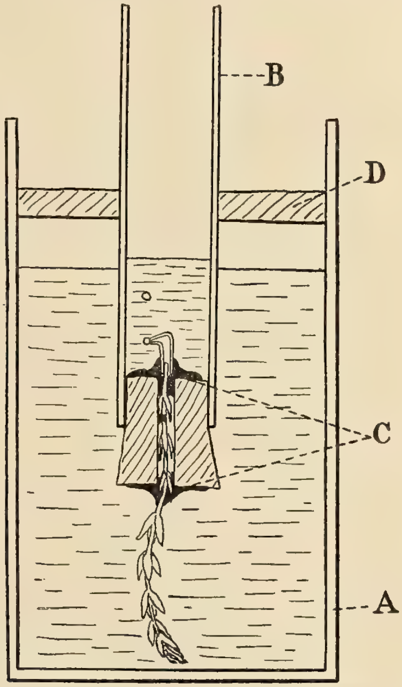

Fig. 1.—Fig. 1 shows the arrangement of the parts during experiments. A, flat-sided glass museum jar, containing the experimental solution; B, "bubbling cup," containing distilled water, closed by cork below; C, wax mixture, which supports the shoot in the cork and completely separates the liquids in A and B; D, cork lid, which hinders the loss of CO_2 from the top of the solution and supports the bubbling cup and also a thermometer. The glass "bubbler" is shown attached to the cut end of the shoot with a bubble escaping from it. [In the figure, to save space, the shoot and the jar, A, are drawn much too small and short relatively to the essential central parts.]

It was demonstrated that the bubbles have a larger volume when the vessel contains osmotically active salt solutions than when water is used. In a whole shoot of Elodea carrying on assimilation in a salt solution the effect would be recorded as a smaller number of bubbles in unit time, which is what Treboux found in his Experiments 1 to 6. On replacing the salt solution by distilled water, the cells, now containing salt, absorb water and swell round the aperture, reducing its size and also that of the bubbles, and sometimes (after strong solutions) completely stopping bubbling by closing up the aperture.

With this apparatus was also demonstrated the effect of changed surface-tension. Thus, on adding methylated spirit to the vessel the bubble size is decreased, which in natural experimentation would express itself as an increased rate of bubbling, such as Darwin and Pertz observed. This smaller size of bubbles on adding methylated spirit was produced (to about 10 per cent.) also when a glass bubbler was fixed to the free end of the Elodea stem, and air forced through the whole system.

The mechanism of bubble-initiation, swelling, and liberation with the glass bubbler, as described above, was followed carefully with this apparatus at various rates, and as produced by different heads of water. In some cases a slight head of water produced no bubbles, but increasing pressure caused a sudden rapid rush of bubbles, which quickly slackened and then died completely away. Further increase caused a repetition of this without any regular bubbling occurring. This irregularity was evidently caused by the formation of water films across all the intercellular spaces, bubbling occurring only when the pressure became sufficient to burst them, after which they re-formed. These same "rushes of bubbles" occurred also with material used in these experiments, seeming to be most frequent during very dull weather. Probably during very dull spring days the respiration may exceed the assimilation, the volume of the internal air system being reduced until water films are formed. Sometimes a plant which at first refused to bubble would begin in the middle of an experiment (two shoots side by side were used to ensure one of them finishing the experiment) and either bubble regularly or in "rushes."

Adopting the bubblers and bubbling cup described in this section, we have every reason to believe that all the sources of error in bubble-counting which are due to variation in size of bubbles have been successfully removed, so that simple counting becomes a direct measure of volume of gas leaving the shoot, even when different plants are used in a series of experiments. There is now another, more subtle source of complications to be considered.

(B) *Method of Diminishing Errors due to Gaseous Diffusion in the Water round the Plant.*

As the gaseous exchange of the assimilating plant takes place in the centre of a mass of water, its supply of CO_2, when the water is not in movement, depends upon diffusion from all around the plant. This factor is particularly important in laboratory experiments with vessels of very still water, and but slight temperature-convection currents. Certain effects due to this cause have not always been rightly understood. Similarly with the oxygen produced: this may leave the plant by the path of diffusion into the water as well as by escape through the intercellular spaces. Both these matters have been sources of illusion in early experiments.

Ba. *Aberrations due to Deficient Oxygen in Solution: the "Initial Oxygen Diffusion Effect."*—The less oxygen there is in solution in the water the greater must be the proportion of photosynthetic oxygen which diffuses away into the water and fails to record itself as bubbles. Most observers have used for their work water in which the oxygen in solution is no greater than that due to equilibrium with the atmosphere, so that the water against the plant only contains initially 21 per cent. of the volume of oxygen it can hold in solution in equilibrium with pure oxygen. In such a medium, when the plant is first illuminated, the bubbling rate is very slow, but steadily increases, more and more of the oxygen escaping as bubbles. The increase is at first rapid and then slows up smoothly towards an approximately constant rate, reached when only a little is passing away by diffusion. Fig. 2 gives an excellent illustration of the effect. These data are taken from Angelstein (p. 97). The nearly level rate that is obtained in due time is not absolutely stable, but depends on a steady diffusion gradient being maintained. Any small mechanical shake is liable to disturb it. Stirring brings the bubble rate down instantaneously and then it slowly climbs up again. This phenomenon has necessarily been encountered by all workers. Some have discarded these initial periods altogether, some recorded them as disturbing factors without explanation, and others have interpreted them correctly, but no one seems to have attempted to eliminate them for their research work, systematically, by charging the solutions to be used with a larger amount of oxygen. In working on the effect of drugs and other solutes on bubbling plants time is precious and the initial period cannot well be spared.

In the work on effects of acids and bicarbonates which follows, water was used which was charged with oxygen by prolonged violent shaking in bottles with an atmosphere of pure oxygen from a cylinder. The charging process is very slow in its later stages. Such water was used for diluting the standard

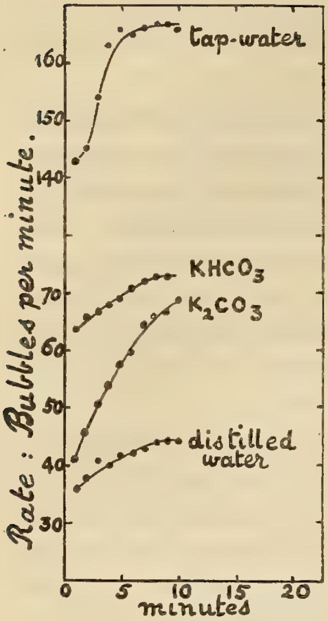

FIG. 2.

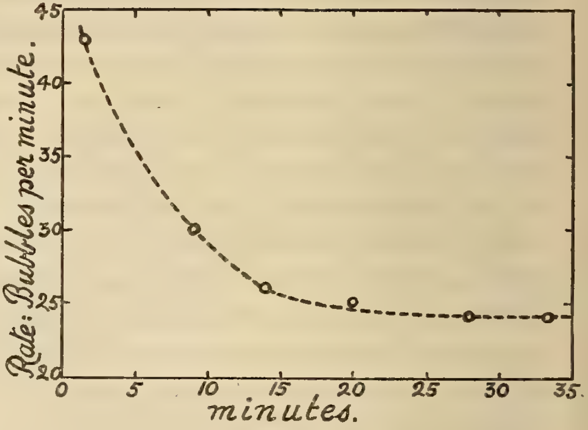

FIG. 3.

FIG. 2.—Initial oxygen diffusion effect plotted from Angelstein's experiments. Four solutions, respectively distilled water, dilute $KHCO_3$, dilute K_2CO_3, and tap water, were allowed to stand in shallow dishes in the air for fourteen days that their CO_2-content might come into equilibrium with the air, and then the rates of bubbling of Elodea were measured in them. It is obvious that during the initial period here figured, which is all Angelstein records, the bubble rates, rising rapidly as they do, do not measure the CO_2-content, but merely the building up of the oxygen diffusion gradients in the solutions.

FIG. 3.—"Initial CO_2 diffusion effect" with Elodea set up in a low concentration of CO_2.

strong CO_2 solutions before use; for the sodium bicarbonate solutions the solid salt was dissolved directly in it. The test of its efficiency was that when bubbling was started no such initial oxygen diffusion effects as are here described were to be observed.

Bb. *Carbon Dioxide in Solution: the "Initial* CO_2 *Diffusion Effect."*—We have now to deal with a second aberration depending on diffusion that characterises the initial stages of bubbling in a solution. When a plant is first put to bubble in a solution containing CO_2 the layers against the plant are charged with this solute to the same extent as the general body of the fluid. As photosynthesis proceeds they are robbed of CO_2 and a diffusion gradient is gradually built up, supplying fresh CO_2 from the more remote layers. The *effective* concentration, then, is never so great again as at the first moment. If the CO_2 concentration is not very great compared with the intensity of the other factors, so that CO_2 is a limiting factor in Blackman's sense, then the photosynthetic rate will necessarily be high at first and steadily decline as the diffusion gradient slackens, until an approximately steady gradient is established, bringing along CO_2 at the rate which is that appropriate to the general concentration. Here we have an initial aberration which is exactly the inverse of the oxygen aberration. This is illustrated in fig. 3, drawn from the present work. This phenomenon repeats itself after every movement or stirring, which brings up more highly charged layers of CO_2 solution, and also after every period of darkening during which CO_2 has again accumulated round the plant. This type of initial effect has also been often observed, but hardly ever precisely interpreted.

It is interesting to note that it only occurs when the conditions of experimentation are such that CO_2 is limiting. When weak light is limiting and the CO_2 concentration is in excess, these variations of CO_2-supply cannot, by definition, manifest themselves as changes of bubble rate, and it is found throughout the present work that the falling initial curve occurs only in the one class of cases and not in the other.

This initial aberration cannot be removed by any preliminary treatment. If investigation is to be made on the relation of assimilation to CO_2 concentration or to $KHCO_3$ solutions, this effect is bound up with the problem stated. The only theoretical way would be to use solutions which were always in violent movement, so that there were practically no layers less charged with CO_2 than their neighbours, but in such cases it would be very difficult to keep the lighting of the shoot absolutely constant.

Bc. *Combination of Initial Oxygen Diffusion Effect with Initial* CO_2 *Diffusion Effect.*—We now see the experimenter is faced with two initial effects of entirely opposite nature, one giving a rising series of readings and the other a falling series. The combination of them suggests interesting complications as each is independent of the other. It would be possible for the two to neutralise one another if the solution started with exactly the right deficit of oxygen and excess of CO_2. It seems highly probable that in the cases here and there in the literature where the bubble rate starts at once at the rate that it continues to maintain we have such a relation. What appears to be an ideal experiment will then be only a chance cancelling out of two unmeasured sources of error.

The experiments in this paper show big CO_2 effects as compared with other workers because special trouble was taken to eliminate the oxygen effect. No one is directly interested in the relation of bubble rate to the oxygen-content of the water, and everyone in its relation to the CO_2-content, so the best procedure seems to be to eliminate one source of error and so get a clear measure of the magnitude of the other source.

In the following experiments the initial CO_2 effect occurred so regularly and was so obvious to the eye that readings were often omitted during it. In the graphs of many of the experiments figured in this paper it will be seen that the curve of the initial phase has been drawn in without any actual data.

At the end of the initial phase (some 10 to 20 minutes) the bubble rates become practically constant for a long time, and it is these long constant values which are taken as the measure of the CO_2 assimilation possible when the CO_2 in solution or the CO_2 of bicarbonates is limiting. When the investigator changes the conditions every 10 minutes, as Pantanelli sometimes did, it is clear that the results recorded may be involved in considerable complications. Earlier observers have tried to rush through their experiments as quickly as possible to avoid spontaneous uncontrollable variations in bubble size, but the use of glass bubblers removes all this risk, and experiments can be carried out slowly and constancy assured in each condition before it is changed.

The uniformity of bubble rate is so regular a happening when a bubbler is used that the observer gets into the habit of taking many fewer readings of bubble rate than would be permissible with a varying cut stem. It will be noticed that in many of the graphs the actual points recorded seem rather scanty, but they are really quite adequate to establish the conclusions drawn when working with this improved technique.

(C) *Apparatus, Experimental Procedure, and Minor Sources of Error.*

The plants used in subsequent experiments were either Elodea or Callitriche. They were fitted with a glass bubbler and set up, depending from the cork of the bubbling cup in the way shown in fig. 1. The bubbling cup always contained distilled water, and it was fitted into a hole in a thick cork plate, which again fitted into the rectangular glass museum cell used as a plant chamber. For single shoots a chamber of 200 c.c. capacity was used. A hole in the plate near one corner, closed by a cork during experiment, allowed fresh solutions to be poured in through a funnel, while a small piece cut from the opposite corner allowed the liquid to be quickly poured away. In the experiments with acid the required amount of N/10 HCl was added through this hole from a graduated pipette, and stirred by twirling a glass rod which passed to the bottom of the cell and had its lower inch bent at right angles and flattened. The temperature could be read continuously from a thermometer passing through another hole in the lid.

The CO_2 solutions used were diluted from a stock standard containing about 12 volumes per cent. CO_2, prepared from a Kipp's apparatus and cleansed from acid in the usual way; the actual strength was determined by titration with excess of baryta and hydrochloric acid. For the avoidance of the initial oxygen diffusion effect solutions were made up as required by diluting this stock with water richly charged with oxygen, as already described. In the preparation of bicarbonate solutions the exact amount of sodium bicarbonate required was weighed out each time and dissolved in the oxygenated water just the moment before it was used.

Many shoots of water plants are curved near the tip. It is important to set up the shoot with the plane of the curvature at right angles to the light rays, so that the distance from the light remains constant after any re-setting of the shoot or alteration of its curvature.

Artificial light is needed for prolonged uniform illumination. To obtain strong enough light some observers have placed cylindrical vessels of water in front of the water-chamber containing the plant. These condense the light, as lenses, into a vertical strip upon the shoot. Experiments with this type of apparatus showed that the rates of bubbling were very sensitive to the slightest displacement of the cylinder or of the plant. A variation of the rate of bubbling by as much as 100 per cent. might be produced by shifting the plant 1 to 2 cm. laterally while the rest of the apparatus was unmoved. Such condensing cylinders were therefore abandoned. The light used in these experiments was that of a cluster of three large incandescent mantles fed with gas under a pressure of 8 inches of water (the Keith light). This gives a radiating surface about 3 inches wide by 5 inches high, and is very suitable and steady. The three burners are grouped on an equi-lateral triangle plan and the distance of the plant from the centre of this triangle is recorded. In some cases, where it has any significance, the intensity of illumination is expressed by calculation on the "inverse square of the distance" basis. The intensity at a distance of 10 cm. is arbitrarily taken as equal to 10 units. This law does not, of course, apply with any accuracy to so large a source of light.

If the temperature of the water and plant rise much during experimentation, errors of various natures, biological and physical, will creep into the readings. It is important to have an effective running water screen in front of the plant chamber to cut out most of the rays absorbable by glass and water, but it is inevitable that the shoot should be somewhat warmed by the additional radiation that it absorbs. In these experiments the experimental solutions did not rise in temperature more than ½° to 1° C. The temperature of all the experiments lay between 15° and 18° C.

The rate of bubbling was timed at intervals with a stop-watch. The number of readings shown on the figures may appear rather scanty, but bubbles came off so regularly and slowly that each point recorded is in reality a series of as many readings as bubbles observed.

Part II.—ASSIMILATION BY ELODEA IN DILUTE SOLUTIONS OF HYDROCHLORIC ACID.

Treboux (1903) tried the effects of many solutes upon the rate of bubbling of submerged plants, and he found that they all had a depressant effect, except one class of substances—the organic and inorganic acids—which increased the bubbling rate considerably. Treboux does not seem to have considered this at all an amazing result, but simply analogises these acids with carbonic acid, which, of course, also produces the same consequence. At the suggestion of Dr. Blackman, I took up this problem to test whether this general action of acids is due to any direct action on the cell, or merely to their setting free carbonic acid from the plant, and so leading to an increased assimilation. Hydrochloric acid was selected for experiment, and it soon became clear that its action is of the indirect nature here suggested. All this work was done in 1911. The only modern comment on Treboux's results that has been noted is by Willstätter (1918, pp. 53–4), who thinks it improbable that they are due to a direct action on the photosynthetic mechanism. He suggests that it is more probable that the acid acts on the CO_2-adsorbing property of living tissues, an effect which he has studied, displacing CO_2, which may increase the bubbling, partly by escaping as a constituent of them, and partly by augmenting assimilation.

Treboux's observations with acids were numerous, and his effect is perfectly well established, though he notes that some other observers have failed to detect it. A single example will illustrate his results (Treboux, Experiment 36). The bubble rate was observed in water containing 0·3 per cent. CO_2, and then increasing quantities of acid were added, the increase of bubble rate being recorded for each stage: finally, the plant was returned to CO_2 solution without acid. The solutions and rates are as follows:—

Concentration HCl in addition to 0·3 per cent. CO_2	Nil	0·0001 N	0·0002 N	0·0003 N	0·0004 N	Nil
Bubble rate per minute	26	46	66	86	106	26

Were Elodea one of those water plants which become in time obviously encrusted with chalk, explanation of the action of acids would be simple, but to the eye there is no sign of such incrustation.

Experimental Results.

Experiments were first made in spring with Elodea that had been growing all the winter in an open wooden tub, the water in which was largely rain water.

Figs. 4 and 5 show that this material does not exhibit the effect described by Treboux. Neither acidification increases the bubble rate appreciably. In fig. 5 (A) the early rate is lower than the later: this appeared possibly due to gas-pressure variation. Such instances of variation of rate without change of conditions were extremely rare: at such times the light did not appear to the eye to be as perfectly steady as usual.

The next step was to obtain Elodea from a natural water, and in April experiments were made with material from a chalky stream (at "Nine Wells"). Here the result is quite different, as figs. 6 and 7 show.

In fig. 6 the addition of acid about doubles the rate of bubbling in B. On setting up the plant in the unacidified CO_2 solution, it returns in D to its original rate, to be again accelerated by acid. At C it is seen that stirring causes a new transient "initial CO_2 diffusion effect," with return to the previous rate. If any of the increased bubble rate in B had been due to making good a previous lack of oxygen in the solution, stirring would have brought on a sudden drop, followed by a rising series of bubble rates, instead of the sudden rise with a falling series, characteristic of the CO_2 diffusion effect.

In fig. 7 we have at first a repetition of the effect of fig. 6. The final part provides a demonstration that the effect of added HCl is really due to liberated CO_2, on applying the theory of limiting factors. If enough CO_2 is present in solution, then the rate of bubbling will be controlled by the light only as a limiting factor, and in such case, by definition, a further addition of CO_2 should not increase the rate of assimilation. If, now, all that HCl does is to generate more CO_2 locally at the surface of the plant, then in such a case, with CO_2 already in excess, addition of HCl should not increase the bubble rate. This is what is found at E. It should be noted that the "initial CO_2 diffusion effect" is also absent in D and E, and is very small, partly controlled by the light limit, at the beginning of B. We get thus another line of proof of the nature of the acidification effect.

We may safely conclude that Elodea, in chalky streams, does become impregnated at its surface with $CaCO_3$, though not enough to be visibly affected, and that, after growing for some time in soft water, the shoots lose this calcification. The Elodea grown in a tub owes its inability to give an "acid effect" to this cause, and it was further proved that, when the material from the chalky stream was placed, in April, to grow in the tub, it ceased after two or three weeks to give this effect any longer (see Experiment table on p. 318).

Some further illustrations of the presence of the acid effect with fresh material when CO_2 is limiting and its absence when light is limiting are afforded by the next two experiments.

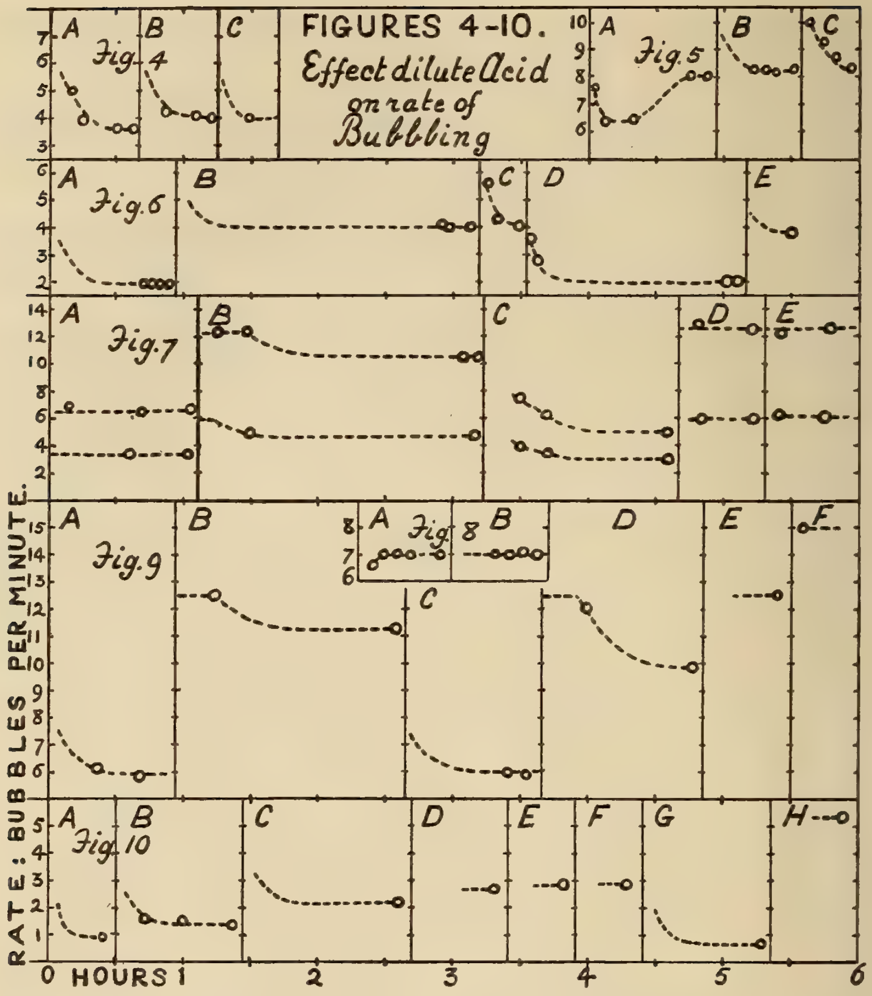

Fig. 4.—Elodea: Light intensity = 6*; temperature 16° C. A, in 1 per cent. CO_2 solution†; B, HCl added to 0·0005 N; C, further HCl to 0·0015 N.

[In this and all other figures the broken line indicates the course of the bubble-rate that is to be assumed where there are no countings given.]

Fig. 5.—Elodea: Light intensity = 7·6; temperature 15° C. A, B and C, as in fig. 4.

Fig. 6.—Elodea from chalky stream: Light = 6; temperature 17·5°–18° C. A, 3·33 per cent. CO_2 solution; B, HCl to 0·0005 N; C, solution stirred but not

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* Light intensity at 10 cm. from centre of burners is called arbitrarily = 10; other intensities were obtained after calculating by inverse square law the distance necessary to give the required value, the light being then set by means of a metre scale fixed alongside the apparatus. Values are thus only roughly approximate.

† All the strengths of CO_2 solution are expressed as volumes CO_2 at 0° C. and 760 mm. Hg contained in 100 volumes of solution.

In fig. 8 the CO_2 supply is well in excess of the light intensity, and no increased bubbling results on addition of HCl. In fig. 9 we have a more elaborate experiment, showing in its first part, sections A–B–C, a large effect of acid when weaker CO_2 is used, and in its second part, D–E, a small effect when stronger CO_2 is supplied; a third part, F, was carried out to demonstrate that it was really the limiting factor of light which kept the effect so small in E, for on simply bringing the light nearer, in F, the rate of bubbling rose at once.

In the next experiment of this series four separate shoots were set up to bubble in one large jar containing 500 c.c. of CO_2 solution; the results of acidification are tabulated below. Plant C came fresh from the chalk stream, A had grown in the water-tub all the winter, while B and B′ were plants from the same habitat as C, but brought in some weeks earlier and transferred to the tub. The bubble rates of the individual plants in the one solution differed a good deal, partly determined by the sizes of the shoots and partly by the different sized nozzles of the four bubblers (C was a very short stout shoot, and its bubbler had a very large opening). To make them easily comparable, the actual rates have been multiplied by a factor, given at the head of the Table, to bring them all to the value of 10 for the original rate in CO_2 solution. On adding HCl to bring the acidity to 0·0008 N, the

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changed; D, solution poured off and a second half of original 3·33 per cent. CO_2 solution put in; E, again HCl to 0·0005 N.

Fig. 7.—Elodea from chalky stream, two plants side by side in the same chamber—upper and lower records. Light = 12·3; temperature 18° C. A, in CO_2 solution; B, added HCl to 0·0010 N; C, second half of solution used in A; D, some strong stock CO_2 solution added to make the CO_2 very strong and superlimiting, in order to determine the light-limit value; E, HCl added to D to 0·0010 N; the light is now limiting and the effect of acid quite absent (see text).

Fig. 8.—Elodea from chalky stream in moderate light and strong CO_2, so that light is limiting. Light = 6; temperature 18° C. A, 3·7 per cent. CO_2 solution; B, HCl to 0·0005 N.

Fig. 9.—Elodea from chalky stream: light intensity 12·3 in series A–E, in F 16. A, 0·65 per cent. CO_2 solution; B, HCl to 0·001 N; C, solution replaced by second half of the original 0·65 per cent. CO_2; D, 1·2 per cent. CO_2 solution; E, HCl to 0·001 N: here the light limit is reached before any large acid effect is manifest: the horizontal line at 12·5 b.p.m. marks the limiting light value; F, the light intensity is increased to 16 without altering the solution, and the bubble rate at once goes up to 15 b.p.m.

Fig. 10.—Elodea from chalky stream: Light = 12. A, in CO_2 solution; then in the five following stages additions of HCl were made to bring the acidity up to the following values; B, 0·00013 N; C, 0·00027 N; D, 0·00054 N; E, 0·0008 N; F, 0·00107 N. In G, the solution was changed for the second half of the solution used in A; the activity is not greatly depressed below the original value in A. In H, a greatly increased concentration of CO_2 was obtained by adding stock CO_2 solution, and the bubble rate goes up enormously.

chalk plant alone gives a positive reaction. It increased its rate fourfold, while the three shoots from the tub showed no increase. It will be seen that, after two hours in acid, the rate of bubbling in A, B, and B′ has fallen to about half. It was not made clear to what extent possible escape of CO_2 from the solution, reduction of CO_2 concentration by assimilation, and injurious effect of the acid, were responsible for this big fall, as there was no opportunity of doing further experiments on this point. It is interesting to note that the extra source of CO_2 available for the chalk plant has come to an end, and that its rate also has fallen to half the original value. The termination of the temporary acid-chalk increase ought to be investigated further.

Experiment Table.

Elodea.	Grown in rain water.			From chalk stream.
4 shoots	A	B	B′	C
Rate factor	7·8	18·3	1·23	85·0
Time.	Relative rates of bubbling.			
p.m.				
12.8	10·0	—	10·0	
12.10	—	10·0	—	
12.13	—	—	—	10·0
12.15	10·0	—	10·0	
12.19	—	10·0	—	
12.20	4 c.c. N/10 HCl added to make acidity 0·0008 N.			
12.35	10·8	—	—	
12.36	—	—	10·2	40·0
12.38	—	11·5	—	
1.5	8·0	—	8·8	
1.9	—	10·0	—	39·2
2.10	—	—	—	5·2
2.12	—	—	6·1	
2.17	—	5·8		
2.23	5·5	—	—	
2.30	—	6·3	—	5·0
2.35	4·9	—	—	

The effect of dosing the plant, as Treboux did, with a series of increasing strengths of acid, is shown in fig. 10. In this case the first two increases of acid each produced a marked rise in bubble rate, but with subsequent increases the further rise diminishes until between E and F it is nil. That the value here reached is not merely a light limit is shown by the greatly increased rate in H when more CO_2 is given. In fig. 11 the rates of bubbling are plotted against concentration of acid, and it is seen that a linearly increasing rate, at first, is followed by a uniform rate so that a curve like a CO_2 light limiting factor curve is produced. Knowing that the light limiting value is much higher, we cannot put this simple interpretation on the result. It must be remembered that it took four hours to carry through the series B to F, so that a time factor of some sort may be responsible for nullifying the direct effect of the stronger acids. It may be noted that in the Experiment table on p. 318 the acidification effect on C had fallen to nil in about two hours time, though this is not usually so. There was no opportunity of continuing the investigation and analysing out the factors that contribute to give the curve of fig. 11 its particular form.

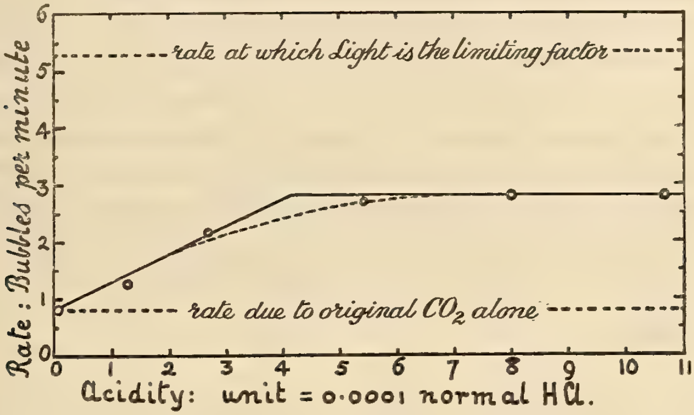

FIG. 11.—Effect of increasing acidity on bubble rate with Elodea. The data are taken from the experiment set out in fig. 10. It will be seen that, at the critical part of the curve, the data are not adequate to determine whether the points lie on a continuous curve or on two intersecting straight lines.

Part III.—COMPARISON OF ASSIMILATION RATES IN SOLUTIONS OF CARBONIC ACID AND OF SODIUM BICARBONATE.

Though several workers have studied the rate of bubbling of water plants in solutions of bicarbonates, no one has compared these rates of bubbling with the rates in known solutions of carbonic acid. With the aid of a bubbler and bubbling cup described in Part I, comparisons between different solutions can readily be made with the same plant, as a uniform bubble size is assured, whether the plant is in a solution of carbonic acid or of bicarbonate.

The results of Angelstein (1911), who observed bubble rates in a solution of bicarbonate which he gradually increased in concentration, are very obscure, especially because in all cases he chose to add CO_2 to the water before beginning the series of bicarbonate strengths.

For determining what strengths of CO_2 solutions are equivalent, in assimilation capacity, with given strengths of bicarbonate, the ideal plan would be, after preliminary experiments, to be able to present results giving identical rates in a pair of selected solutions. Only a short time was, however, available for the experimental work of this section, and the equivalence of pairs of solutions had to be deduced by rule of three. For the soundness of this procedure it must first be demonstrated that in a series of CO_2 solutions of different strengths the rate of bubbling is directly proportional to the concentration within the range that it is proposed to use; the same proportionality should also be demonstrated for a series of bicarbonate concentrations. These two demonstrations are given in the next two sections, and the direct comparisons of CO_2 and bicarbonate follow in a third section.

(A) *Experiments with Carbonic Acid Solutions of Different Strengths.*

The results of the first experiment, in which the solution in the plant chamber was changed frequently for others of different strengths, is given in fig. 12. Each new solution shows the "initial CO_2 diffusion effect"* leading to a steady rate. This steady rate in each solution would be maintained for

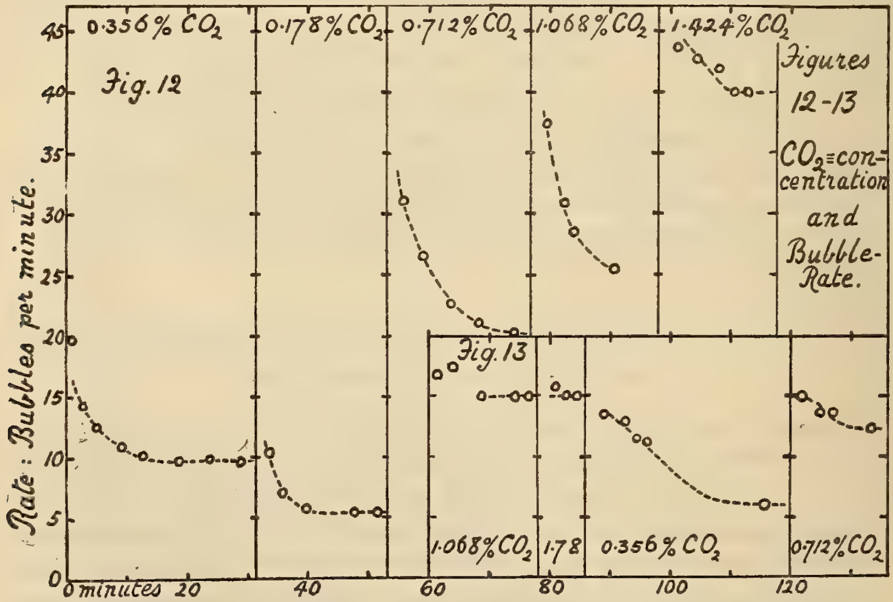

FIG. 12.—Bubble rates of Callitriche in a quickly changed series of CO_2 solutions, showing the initial CO_2 diffusion effect before the bubbling settles down to a steady rate. Strong light (= 10); temperature 17° C.

FIG. 13.—Similar series to that in fig. 12, but with a weak light of 3·5 units. Light now limiting in the first two solutions.

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* See Part I, p. 310.

a long time, but readings were not continued in this particular experiment beyond the point necessary to determine what this steady rate was for each solution; for even thus the experiment occupied two hours. The final values are given in fig. 14 plotted against CO_2 concentration. They suffice to demonstrate that the bubbling rate is directly proportional to the concentration. This is not a new point, so that no elaborate experiments were made. Treboux demonstrated it, and it accords with the findings of Blackman and Smith (1911) using CO_2 analysis instead of bubble rates. It must be noted in fig. 12 that one of the values, the rate in the solution believed to be 1·068 per cent. CO_2, is quite out of proportion by some error or other, but it was not thought worth while to repeat the series. In this experiment a strong light (intensity = 10) was used, and the light was in excess throughout. In the next series the light was reduced to 3·5 units, and a similar range of strengths of CO_2 was repeated.

These results are set out as a time sequence in fig. 13. In the last two strengths we have the same initial effects as with the strong light series, and here CO_2 is still limiting. In the first two strengths the CO_2, however, is in excess, and the light limiting, and after some irregularities the final rate is

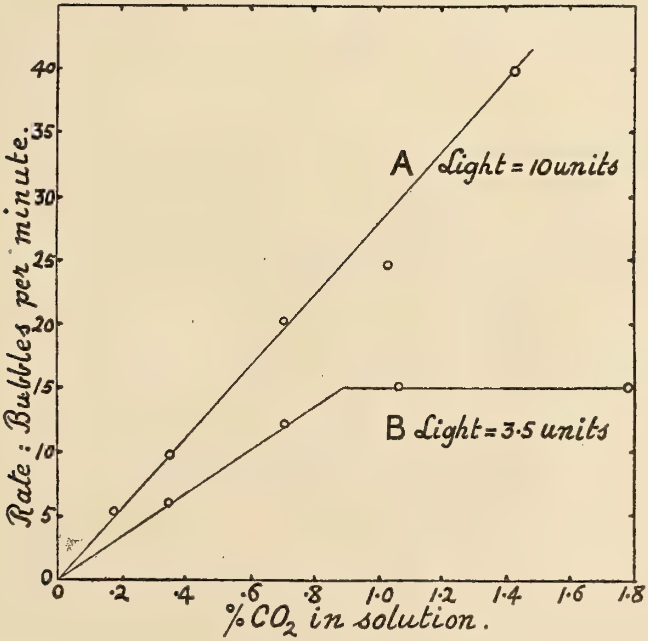

FIG. 14.—The final values adopted from figs. 12 and 13 are here plotted against CO_2 concentration. With weak light (3·5) the proportionality between concentration and bubble rate is cut off at about 0·9 per cent. CO_2 by the light factor, while with strong light (= 10) this proportionality is continued through the whole series tried.

attained without the falling curve. Here the CO_2 initial diffusion effect cannot reveal itself. The experimental record given is slight and brief and is only intended as an illustration. Throughout the work the relations of the factors light intensity and CO_2 concentration conformed to Blackman's exposition of the theory of limiting factors.

The final values for the four concentrations of fig. 13 are transferred to fig. 14, lower curve B, and it will be seen that they exhibit the form of the two-factor limiting curve. With weak light and strong CO_2 Treboux also records this type of curve.

This demonstration may suffice to establish that for our experiments, comparing CO_2 and bicarbonates, it is accurate to calculate bubble rates from one strength of CO_2 to another on the basis of direct proportionality; provided, of course, that care is taken to ensure that light is in excess throughout.

(B) *Experiments with Sodium Bicarbonate Solutions of different Strengths.*

Experimenting with series of bicarbonate solutions one does not encounter such simple regularity as with CO_2 solutions. After the initial CO_2 diffusion effect, which occurs here also, the rate is not maintained uniform for such long periods, but gradually declines. This is presumably to be attributed to the local alkalinity of the solution that must result from the removal of CO_2. Angelstein records the injurious effect of added Na_2CO_3 in increasing the alkalinity, but this effect was not detached from other complications. Escape of CO_2 from the upper surface of the solution would increase the alkalinity of the liquid, so for the more precise experiments of the next section (C) a cork lid was employed to retard this effect. For the present section we have two experimental series, each of five increasing strengths of bicarbonate. At each strength of solution the plant and chamber were carefully rinsed, to remove adherent alkaline liquid. Such a series cannot be carried through in less than two hours, so to reduce the duration of the exposure, the bubble rates were only followed just to the end of the initial CO_2 diffusion effect in each solution. The final value adopted for each solution is not therefore as firmly established as if a long series of similar values had been counted. The time-sequences of these experiments are not detailed here, but the final adopted values for the two series are given in fig. 15. Both Series A and B were carried out with weak light (intensity = 3·5), but A with such weak bicarbonate solutions that the light limiting value was not reached. The points for Curve B, which is more extensive, are not very smooth, but the general relation of two limiting factors reveals itself here, as with carbonic acid. The data for A only presents the rising limb of the curve; presumably, had stronger solutions been used no higher bubble rate would have been recorded. The points for A make a smooth sequence, but do not lie in a straight line: as the concentration increases, the bubble rate declines somewhat from direct proportionality. Whether this is a primary relation or is only an expression of the inflection of the curve, at the highest value, into its horizontal course has not yet been investigated.

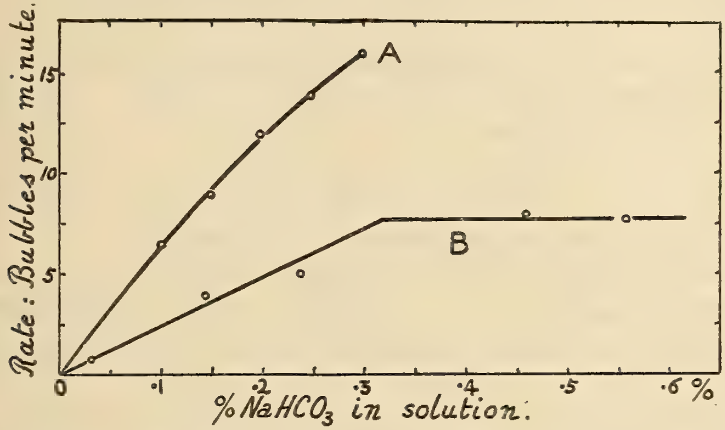

FIG. 15.—Rate of bubbling in sodium bicarbonate solutions of different concentrations. For both curves, light = 3·5. Curve A, one plant in five solutions of increasing strength; Curve B, another plant in five solutions covering a wider range. B shows a typical limiting factor curve, but A stops just where the horizontal limb would be expected.

Figs. 14 and 15 together enable us to compare the strengths of carbonic acid and sodium bicarbonate that *just* suffice to give the full effect that is potential in light of 3·5 units. These comparable values are given by the inflection points of the two B curves, both of which are for 3·5 light. Whereas 0·9 volumes of CO_2 per cent. is the critical value for CO_2, it is seen that 0·32 grm. $NaHCO_3$ per cent. is the corresponding value for bicarbonate. This provides us with our first equivalence ratio, $\frac{\text{ctn. bicarbonate}}{\text{ctn. } CO_2} = 0{\cdot}35$. This comparison is made very indirectly with two different plants at different times, and the location of the supposed inflection point is only got by interpolation. Direct comparisons set out in the next section show that 0·29 is a better value for this ratio.

The experiments described in this section, though not very searching, suffice to establish that for different concentrations of bicarbonate the bubble rates, if not in absolute direct proportion to the concentration, approximate closely to this relation, especially over a short range of concentrations.

(C) *Direct Comparisons of Sodium Bicarbonate and Carbonic Acid with the same Shoot.*

For the experiments in this section the rate of bubbling was first taken in CO_2 solutions, and then the plant was transferred to a bicarbonate solution. This sequence gives no risk of the readings in CO_2 containing an injurious after-effect of the bicarbonate solution. Fig. 16 shows the readings of the first of these experiments. Each fresh solution shows its "initial CO_2 diffusion effect," leading to the final value representing the static efficiency of the solution. A and B give concordant values, 2·4 b.p.m. in 0·6 per cent. CO_2,

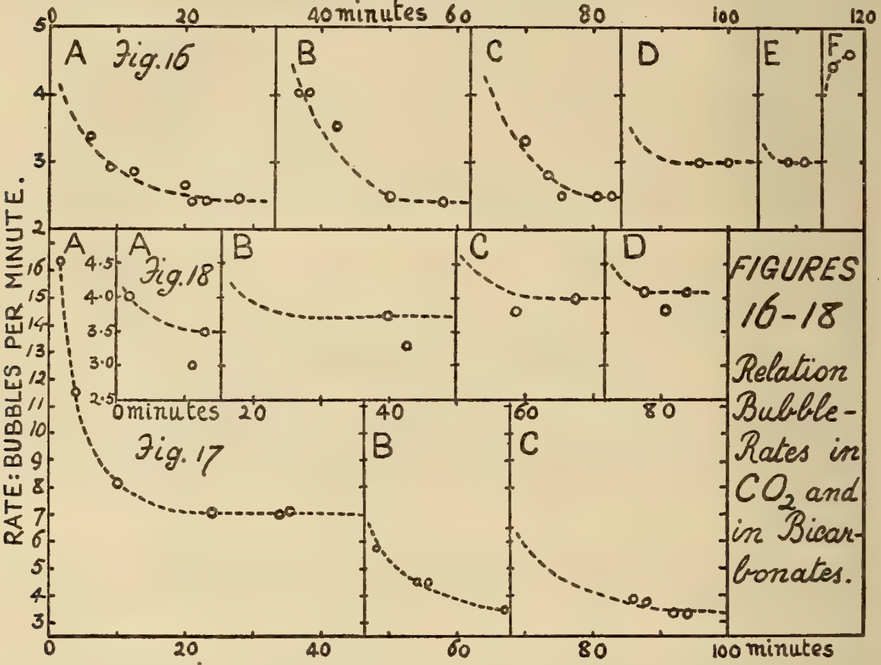

Fig. 16.—Bubble rates of Callitriche in six successive changes of solution. A, 0·6 per cent. CO_2 solution; B, fresh 0·6 per cent. CO_2 solution; C, 0·2 per cent. solution of $NaHCO_3$; D, fresh solution of the same concentration; E, ditto; F, 0·9 per cent. CO_2 solution. This last stronger solution was only employed to demonstrate that the values A–E are none of them limited by light.

Fig. 17.—Bubble rate of Callitriche. A, 0·6 per cent. CO_2 solution, the plant being observed for a long time in one solution instead of renewing the solution; B, 0·08 per cent. $NaHCO_3$ solution; C, fresh ditto.

Fig. 18.—Bubble rate of Callitriche. A and B, 0·6 per cent. CO_2 solutions; C and D, 0·166 per cent. $NaHCO_3$ solutions. This record shows the irregularity that may be got if the bubbler is not absolutely clean, so that some bubbles tend to stick on to the bubbler and increase to an abnormal size before breaking away. In such a case the resulting slow rates are neglected and the quicker values only adopted. This sticking recurs in each solution of this experiment.

but C and D for 0·2 per cent. bicarbonate gave 2·5 and 3·0 b.p.m. respectively. A third sample, E, agreed with D in giving 3·0 b.p.m., and this rate, or the average of 2·8 b.p.m., may be adopted for 0·2 per cent. bicarbonate.

In fig. 17 are set out the data in evaluating the effect of a weaker bicarbonate solution. The rate in 0·6 per cent. CO_2 is 7·1 b.p.m., while in 0·08 per cent. $NaHCO_3$ it becomes 3·3 b.p.m. Fig. 18 presents the results with another strength of bicarbonate. Here duplicate solutions were used and the rates adopted were the quicker rates in B and D, namely, 3·8 b.p.m. in 0·6 per cent. CO_2 and 4·1 b.p.m. in 0·166 per cent. $NaHCO_3$.

The bubble rates established for these three pairs of solutions are set out in the adjoining Table, as well as the adopted rates from two other experiments, which were rather irregular in some details.

The first use we can make of this set of ratios is to calculate from the CO_2 solutions what should be the CO_2 concentrations in the bicarbonate solutions, assuming that direct proportionality holds between rate and concentration in CO_2. For the first case, the calculated CO_2 concentration of the bicarbonate solution equals $0·6 \times 3·3/7·1 = 0·28$ per cent. CO_2. The similarly calculated values for all the other cases are set out in column 6. On this follows the question whether the CO_2 concentrations thus indicated biologically agree with the data derived from physico-chemical work on bicarbonates. Information on this point I owe to Dr. F. F. Blackman, who is dealing with this matter in another paper; he has supplied the numbers of the last column in the Table. These represent the extreme possible values of the CO_2 concentrations in the freshly made bicarbonate solutions used in these experiments. The ambiguity in these values is due to some of the constants involved in the calculation being insufficiently determined.

Experiment.	Assimilation in CO_2 solutions.		Assimilation in bicarbonate solutions.		Estimated CO_2 concentration percentage in bicarbonate solutions.	
	P.c. concentration.	Bubble rate.	P.c. concentration.	Bubble rate.	By assimilation rates.	Physico-chemically (extreme values).
a	0·60	7·1	0·08	3·3	0·28	0·24–0·28
b	0·30	0·5	0·10	0·5	0·30	0·31–0·36
c	0·385	4·0	0·10	3·5	0·34	0·31–0·36
d	0·60	3·8	0·166	4·1	0·65	0·53–0·60
e	0·60	2·4	0·20	3·0	0·75	0·64–0·72

It will be seen that biological results align quite well with the physico-chemical, being close enough to establish the view that the rate of assimilation and bubbling in dilute bicarbonate solutions is due entirely to the concentration of carbonic acid that arises spontaneously by decomposition of the dissolved salt. There is no support for Angelstein's view that such plants actively decompose bicarbonates.

SUMMARY AND CONCLUSIONS.

Part I.—A glass "bubbler" has been devised which, fitted on to the cut stem of submerged water plants, secures that the bubbles liberated in assimilation are of constant size, thereby doing away with one of the chief sources of trouble with this procedure. When, in addition, this bubbler delivers bubbles into an isolated "bubbling cup" of distilled water, the bubbles are removed from the direct influence of the solutions that are being experimented with (see fig. 1, p. 307). In this way alterations of surface-tension by added solutes are eliminated, and any osmotic effects on the cells of the cut surface do not change the size of the bubbles.

The disturbances of bubbling rate due to the "initial oxygen diffusion effect" and the "initial CO_2 diffusion effect" are explained and illustrated. The former (see fig. 2, p. 310), due to inadequate oxygen content of the solution, is of widespread occurrence with other workers, but is eliminated in this work by using water heavily charged with oxygen. The CO_2 effect is the cause of the phenomenon of initial high bubbling rates which rapidly decline (see fig. 3, p. 310). It marks the initial stage before a steady static diffusion gradient of CO_2 is set up. This effect cannot be eliminated when CO_2 is a limiting factor, but does not appear if light intensity is limiting. As these two initial diffusion phenomena are independent and of opposite effect, they may in some cases mask one another mutually, and give a record which does not reveal the initial disturbances.

Part II. Action of Acids upon Assimilation by Submerged Water Plants.—It is shown that the augmentation of bubble rate which Treboux has recorded on adding acid is due to the acid setting free CO_2, locally, from calcium carbonate incorporated in the surface of plants growing in chalky waters. No such acid effect is shown by the same plants when grown in soft water.

That the action of the acid is merely to set free an additional supply of CO_2 is proved by the fact that, with plants grown in hard waters, acid only produces an increase of bubble rate when CO_2 supply is the limiting factor to the rate of bubbling at the time the acid is added. If the plant is placed in relatively strong CO_2 and weak light, so that light is limiting, the addition of acid has no effect upon the rate of bubbling. This evidence seems to dispose of the suggestion that increased bubbling rate with acid is due to CO_2 liberated from the adsorbed state and escaping as a CO_2 addition to the volume of the bubbles. This type of effect should not disappear when light is limiting.

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Part III. Comparison of Assimilation Rates in Solutions of Sodium Bicarbonates and of Carbonic Acid.—By the aid of the "bubblers" described in Part I these rates are carefully compared for solutions of known strength. It is shown that a solution of a bicarbonate gives just about that rate of bubbling which corresponds to the CO_2 concentration expected to arise in it by spontaneous decomposition. Solutions of bicarbonates, when very dilute, give initial CO_2 diffusion effects, conform to Blackman's laws of interaction of limiting factors, and generally behave as solutions of carbonic acid. Angelstein's view that plants have the power of splitting bicarbonates actively is erroneous.

In conclusion I wish to express my thanks to Dr. Blackman, both for advice during the course of the experiments and for help in preparing this paper when stress of official duties, preventing me from giving the necessary attention to it, had resulted in an indefinite postponement of publication.

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Observations on Reflex Responses to Rhythmical Stimulation in the Frog.

By Kanshi Sassa, M.D., Tokio.

(Communicated by Prof. C. S. Sherrington, Pres. R.S. Received July 1, 1921.)

(From the Physiological Laboratory, Oxford.)

The primary object of this investigation was the study of the relation between the frequency and intensity of stimulation, and the resulting reflex reactions. I first studied the effect of alteration of frequency at a given intensity of stimulation and found that under these conditions the frequency has an optimal value. With moderate and rapid frequency stimuli there is also an optimal intensity value, though with those of low frequency this is not the case.

It was also hoped that some light might be thrown on the mechanism of the spinal reflex centre by the comparison of the isometrically recorded reflex tetanus, with the tetanus obtained directly by stimulation of the efferent nerve at varying intensity and frequency of stimulation. In particular, an attempt was made to settle the question as to whether stimulation of a nerve can set into action the whole of the reflex centre to which it is afferent. Camis (4) concluded that the cells of a spinal motor centre can be regarded from a functional point of view, as divided into several independent groups, though this division is not absolute. On the other hand, Dreyer and Sherrington's (5) observations point rather to the physiological unity of the spinal motor centre; since they showed that the maximal mechanical power of tetanic contraction, obtainable from a muscle under spinal reflex action, is sometimes as great as that which can be evoked from it by direct faradisation of the motor nerve itself.

Method.—The semitendinosus reflex preparation of the spinal frog was used in these experiments. The femoral vessels were ligatured in the middle of the thigh so as to diminish loss of blood due to the operation. An afferent nerve, the ipsilateral tibial, peroneal or sciatic nerve or sometimes one of their small branches was employed. When reflex and direct tetanus were compared, the roots of the sciatic nerve were previously exposed by removing the musculature lateral to the urostyle.

To record the muscular contraction, the lower portion of the semitendinosus was isolated from the surrounding muscles, its tendon being fixed to the short arm of a spring myograph. All the tendons of flexors and adductors inserted around the knee-joint were detached, and sometimes the leg was disarticulated at this joint. The pelvis and femur of the frog was firmly fixed by pins to a cork-plate so that the movement of other muscles of the body might not add to that of the semitendinosus. The whole preparation was enclosed in a moist chamber.

The stimulating apparatus was an inductorium fed by two Daniell's cells. To vary the rate of stimulation the "double wire torsion key" devised by Sherrington* was used. The range of alteration of vibration-frequency of the instrument was between 15 and 88 per second. Throughout the experiments only break-shocks were employed. Care was taken that the cathode of the electrodes was proximal to the anode on the afferent nerve for a reflex, and distal on the motor nerve for a direct stimulation. The frogs were kept over-night in the laboratory before being used for experiment; and the time which elapsed between the decapitation and the commencement of the experiment was usually an hour.

I. *The Influence of Stimulation-frequency upon the Reflex Reaction.*

The summation of stimuli in the reflex centre causes increase in the resulting reflex with increase in the rapidity of the sequence of excitation. Stirling (16) pointed out that when a reflex movement is produced in the frog by stimulating a skin point by successive stimuli—the greater the interval between them the higher is the intensity of the individual stimuli necessary to produce the reflex result, and that increase in frequency is more effective than increase in intensity. In the reflex excitation of automatic centres—for example the vasomotor centre—the reflex effect increases with increase in the frequency of stimulation up to the rate of 20–25 per second (11). Matthæi (14) has recently found that the effective minimal number of stimuli, which just produces a reflex reaction, increases in the majority of cases with the frequency of stimulation, occasionally diminishes at first, then reaches an optimum (frequency of 6–21 per second), and finally increases with the stimulation-frequency. In recording my experimental results I will first present the evidence for an optimal frequency of stimulation, by which is meant the rate of stimulation at any given intensity, which produces the most powerful reflex contraction.

1. *The Optimal Frequency-rate of Stimulation.*—In order to determine this, an afferent nerve was stimulated at fixed intensity but at different frequency-rates, and the series of reflex contractions of the semitendinosus thus evoked was recorded isometrically. The intensity of stimulation selected was usually the optimal for a moderately rapid frequency (see later). The lowest rate of excitation was applied for a time sufficiently long to allow the production of

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* A description of this key will appear shortly.

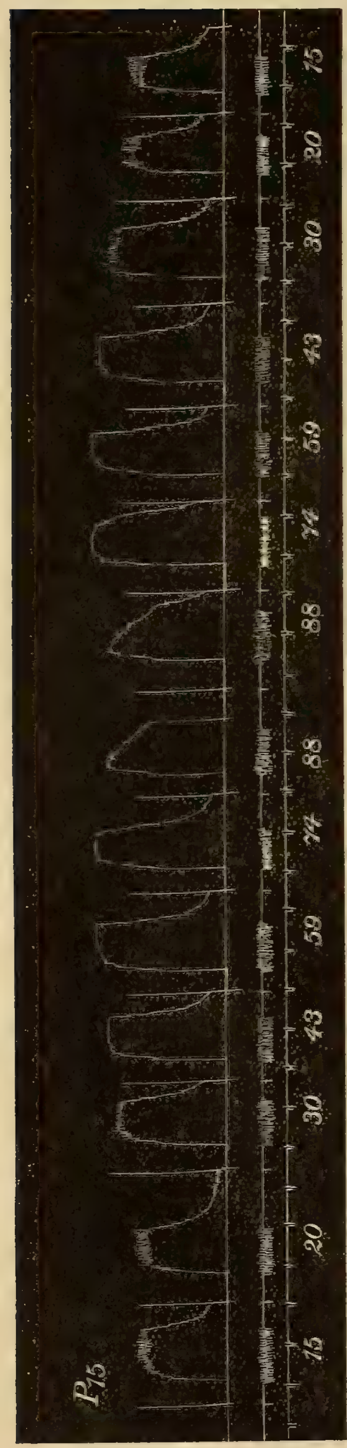

Fig. 1.—Semitendinosus reflex-preparation of spinal frog. Responses at varying frequency of stimulation of peroneal nerve. Distance of coils 15 cm. Threshold value for stimulus, 21 cm. Number of stimuli per second below each response. Time in seconds.

the full contraction it could produce; then, after an interval of about two minutes a somewhat greater rate of stimulation was tried. In this way a series of reflex myograms was obtained at progressively increasing rates, until the maximal frequency which the key could give was reached. This procedure was again repeated in the reverse direction, *i.e.*, progressively decreasing the stimulation-frequency to the initial rate.

The threshold value of faradic stimuli which evoked a reflex response in good preparations was given by a distance between the coils of 20–35 cm., while that for single induction shocks was 15–25 cm.

The strongest reflex contraction was in most cases obtained at a stimulation-frequency of 40–60 per second. In fig. 1 it is seen that the stimulation-frequency of 59 per second is optimal; the reflex reactions given at quicker or slower rates than this are less powerful as compared with that given at the optimal rate of stimulation. The optimal frequency-rate of stimulation is found to be the same if it is tested in the reverse direction, beginning from the highest rate, and gradually decreasing to the lowest.

It must be remembered that the power of reflex reaction diminishes progressively in a preparation which is used for a prolonged experiment. This is due mainly to the impairment of the central nervous system, as shown by the gradual subsidence of reflex excitability. This change does not depend on any damage to the peripheral mechanism, since direct stimulation of the motor nerve gives an undiminished response, and shifting the electrodes centralwards on the afferent nerve or elicitation of the reflex by other afferent nerves does not notably affect the degree of impairment. Local damage is more pronounced when a smaller afferent nerve is used. Even when the diminution of reflex excitability is marked, the optimal rate usually remains the same. In fig. 1 there is no sign of such change of excitability throughout the experiment.

(2) *Mechanical Reflex Rhythm with varying Frequency of Stimulation.*—Various results have been obtained by earlier workers in regard to the relation between the frequency of rhythmic stimulation of the afferent nerve and that of the impulses sent out from the spinal centre to the motor organ. Some investigators have shown that the central nervous system tends to reproduce an intrinsic rhythm of innervation, *i.e.*, about 20 per second (Kronecker and Stanley Hall and others), or about 10 per second (Horsley and Schäfer and others), irrespective of the rhythm of excitation applied to an afferent nerve or to the central nervous system itself. Von Limbeck (12) has, however, found that both in warm-blooded and cold-blooded animals, on artificially stimulating the brain or spinal cord by induction currents, the number of muscular vibrations follows the rhythm of stimulation within wide limits. In the frog and toad the contraction of the gastrocnemius muscle, evoked directly by excitation of the spinal cord, or reflexly by stimulation of the sciatic nerve at a frequency-rate of 13 per second, shows the same rhythm as that of stimulation. He notes besides that, at slower rates of excitation, the mechanical vibrations are double the frequency-rate of stimulation, both make and break shocks taking effect. By a more delicate "resonance method" it has been recently shown by Dreyer and Sherrington (5) that the myograph of the reflex contraction in cats exhibits a mechanical rhythm synchronous with the stimulation at rates up to, and even beyond, 55 a second.

In order to re-examine this point in frogs, a myograph recorder, whose vibration-frequency was 30 per second, was employed. The torsion key was placed upon another table, so that the direct mechanical influence of the vibration of this instrument on the recording lever might be avoided. By this resonance method, the reflex myograph of the semitendinosus evoked by stimulating an afferent nerve shows in the majority of cases more or less clear mechanical tremors synchronous with the frequency of stimulation, whether the rate is 15, 20, or 30 per second (figs. 1 and 2). Further increase of frequency of stimulation up to 40 per second is sometimes followed by the same rhythm of reflex contraction. The reflex centre discharge appears to follow the successive volleys of centripetal impulses at the same rate as those evoked in and transmitted to it by the afferent nerve. The above results in frogs are therefore in agreement with those latest observations referred to above, and are confirmed by investigations made with the string galvanometer (1, 6, 10).

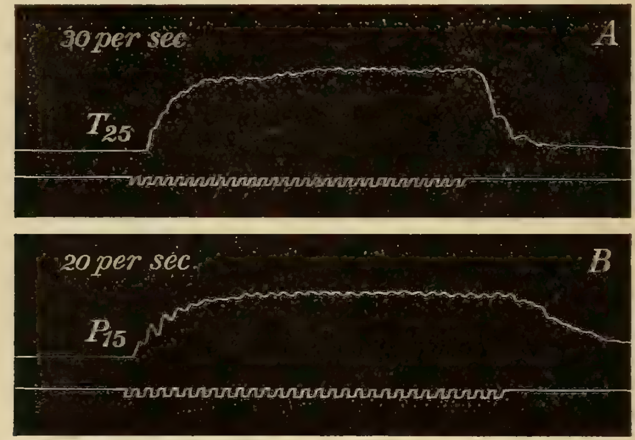

Fig. 2.—Semitendinosus reflex-preparation of spinal frog. The mechanical vibration of muscle synchronous with stimulation frequency. A = tibial nerve; B = peroneal nerve; Number = distance between coils.

When the rate of excitation is low, *e.g.*, 20–30 per second, I have frequently failed to show the synchronism of reflex response with that of the exciting stimuli, though this is clearly evident in the direct tetanus produced by stimulating the motor nerve to the muscle. This is often the case when the excitation at low frequency gives a comparatively high reflex contraction, and in some preparations where the reflex reaction is irregular. The synchronism between the frequency of stimulation of a skin spot of the hind limb and the rhythm of the resulting reflex reaction is soon abolished owing to the irregularity of the reflex response.

(3) *The Course of Reflex Contraction Produced at various Stimulation-Frequencies.*—The course of reflex tetanus is both irregular and variable, at whatever frequency and intensity of excitation it is evoked, as compared with that of direct muscular tetanus. Nevertheless, in typical records some relation between the course of reflex tetanus and the rate of stimulation-frequency which produces it, is usually observed.

With low frequency of stimulation (below 30 per second) it is noted that the reflex contraction, after rapidly attaining its maximum, tends to persist. Often one or two minutes' excitation does not reduce the average height of reaction, though irregular undulations may be seen in the course of the reflex tracing. The spinal centre excited reflexly at this rate does not appear to be easily fatigued, especially since the contraction is not maximal and time is allowed for recovery during the intervals between stimuli.

At a moderate rate of stimulation (40–60 per second), the typical myogram has a smoother and less undulating form (figs. 1 and 4, D). It attains its maximal height rapidly, and maintains it as a smooth plateau so long as the stimulation continues. If the excitation is prolonged beyond a certain duration the height of the reflex contraction gradually decreases.

At rapid frequency rates the reflex myogram is again irregular. The initial contraction subsides rapidly, especially if the intensity of the stimuli is high (fig. 1). This phenomenon recalls the so-called "initial twitch," which was first described by Bernstein (2) in direct tetanus produced by induction shocks of rapid frequency. The similar "initial reflex tetanus" was noted by Fröhlich (8). At the optimal intensity of stimulation for this high rate the myogram is often quite regular, though fatigue easily occurs. In general, a smooth and regular course of reflex response is associated with a powerful contraction.

The results of my experiments are concordant with those obtained by the study of the action current waves of muscles in reflex reaction, in that the central reflex discharge is optimal, when the spinal reflex centre receives impulses from an afferent nerve at the optimal rate. Though the muscular electrical waves in the reflex tetanus are synchronous with the stimulation rhythm up to 100 per second, in summer frogs (Hoffmann (10), Beritoff (1)), the rhythm at which the reflex centre tends to react may be about fifty per second, and the reflex response at this rate is fairly regular and powerful, as judged by the amplitude of deflection of the galvanometer string (Hoffmann (10)). In my experiments, the optimal rate of stimulation, which averages about fifty per second, gives a smooth and powerful reflex response. The irregularity and diminished height of contraction at frequency rates of stimulation higher than the optimal are in accordance with the observation that the muscular action current waves in reflex responses at rapid frequency present irregularity and rapidly diminishing rhythm.

II. *The Influence of Strength of Stimulation upon the Reflex Reaction.*

The reflex contraction elicited by faradisation at or little above the threshold value is inconstant in regard to the height and progress of contraction. The threshold value itself is continually altering, even in good preparations, though within small limits. When, however, the stimulation is strengthened to a certain intensity, the reflex reaction becomes constant in height and course. A definite relationship exists between the intensity at different frequency-rates and the extent of the reflex reaction obtained.

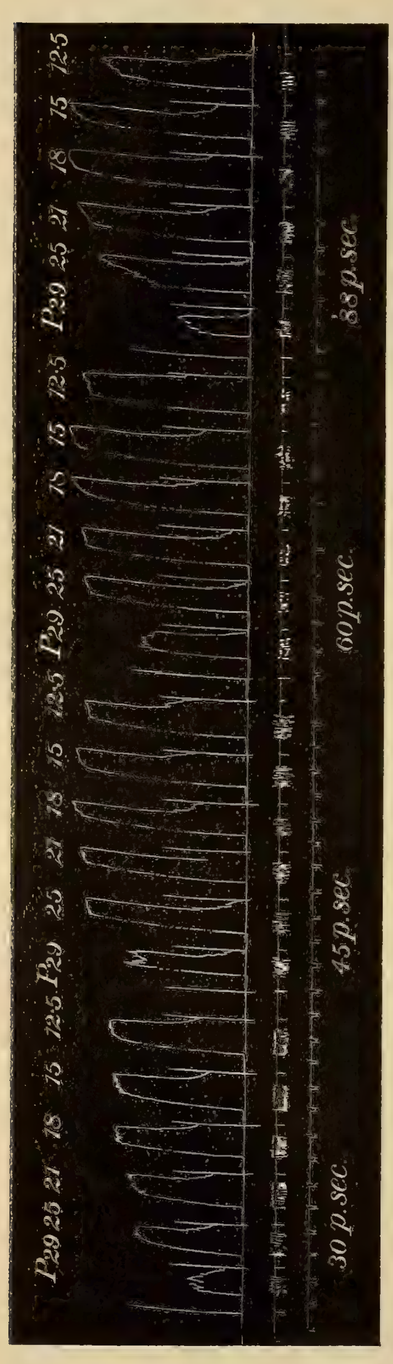

Fig. 3.—Semitendinosus reflex preparation of spinal frog. Groups of responses with frequencies of 30, 45, 60, and 88 per second. The numbers above each response = distance between coils. Time in seconds.

Two methods were adopted of examining this point. In both, several series of reflex responses were recorded. In the first, the frequency was fixed for each series, and the intensity varied within it. In the second, the intensity was fixed for each series, and the rate of stimulation varied.

(1) *Frequency Fixed, Intensity Varied.*—With low rates (under thirty per second), the reflex reaction increases with the intensity of stimulation, at first quickly and then more slowly. Sometimes an intensity is reached, after which no further increase of contraction can be elicited by further increase in the intensity of stimulation. At higher frequency-rates (more than sixty per second), the reflex contraction increases rapidly with the strength of stimulation to a maximum, and from this point decreases with each increment of stimulation. An optimal intensity of stimulation therefore exists at this frequency-rate. Between these two extremes there is a critical value of the frequency-rate, at which the height of contraction remains almost constant after quickly attaining its maximum, in spite of steady increase of intensity of stimulation. In other words, the strength of the reflex reaction under this optimal rate of frequency is independent, within wide limits, of the strength of the stimulation

(figs. 3 and 5). The critical value of stimulation-frequency often coincides with the optimal rate, and is approximately constant in any preparation, as long as this remains in good condition without serious change of reflex excitability.

(2) *Intensity Fixed, Frequency Varied.*—In this method the optimal rate of stimulation in each series is found. If the amplitude of any reflex reaction in one series is compared with the corresponding one in a series obtained at lower intensity, it is found that the reflex response at low frequency is less powerful with low intensity than with higher, while the opposite is the case with greater frequency of stimulation. There has been a shift of the optimal frequency from a lower value at higher intensity to a higher one at lower intensity. Therefore, if the optimal intensity of stimulation for the highest frequency employed has been found, and a series of reflex reactions is registered at this intensity and at progressively increasing rates, the amplitude of reflex response is not infrequently found to increase to the maximum with increase of stimulation-frequency.

The presence of an optimal intensity of stimulation is not limited to very rapid frequency-rates. With moderate frequency it is usually observed, that by increase of intensity beyond a certain value, the reflex reaction thus evoked is less powerful, though the decrease is not so obvious as at higher frequencies (fig. 3). This phenomenon is more marked in some preparations, particularly in those which are impaired by prolonged experiment or are observed during the cold season. Even with a frequency as low as thirty per second, an optimum value may sometimes be obtained if a wide range of intensity is employed. Generally, the higher the stimulation-frequency, the more evident is the existence of an optimal intensity. I have several times observed that the optimal frequency is lower in winter frogs than in summer; in such cases the optimal intensity is more marked with comparatively lower frequencies.

In speaking of an optimal rate of stimulation, it might seem more reasonable to limit the term to the value obtained at the optimal intensity. The latter value is, however, dependent upon the frequency at which it is elicited. Sometimes the reflex response obtained at the optimal intensity at rapid frequencies is equal to, or even greater than, that obtained at the optimal frequency (fig. 3). The contraction has in these cases a smooth and regular course. This optimal intensity of stimulation is evidently of central origin, since a series of direct neuromyal tetani, produced with progressively increasing intensity of faradisation, presents no evidence of the existence of optimal values, except at frequency-rates considerably more rapid than those which exhibit it in the reflex observations (see later). The degree of peripheral tetanus produced increases with the strength of stimulation, until the maximum is obtained for any given frequency-rate, and beyond this point there is no decline, but an unvarying response is given with progressively increasing stimuli.

The experimental results here described concerning the influence of intensity and frequency of stimulation upon the reflex response are in accord with the observations of Wedensky (19), Hoffmann (9) and others, on peripheral tetanus, usually spoken of as "Wedensky's phenomena." When the muscle is tetanised from its motor nerve with strong induction shocks of considerable frequency it relaxes more or less quickly. If the stimuli are then weakened to a certain extent the muscle again falls into a powerful tetanus (Optimum der Reizstärke). If, however, they are again strengthened it enters into its former relaxed condition (Pessimum der Reizstärke). A similar result is obtained by altering the frequency at high intensity of stimulation. For the fresh muscle of the frog, the optimal frequency of stimulation, at which the highest contraction is obtainable in the shortest time, is about 100 stimuli per second (60–150). When lower frequency (about twenty per second) or higher frequency of stimulation (over 260 per second) is used the muscle never attains its maximal shortening in any phase of tetanus. If the preparation is in an unfavourable condition as a result of fatigue or poisoning by ether or curare the optimal frequency of stimulation is lower and the optimal intensity is obtained at lower frequency. On comparing the results in peripheral and reflex tetanus under normal conditions, the most noteworthy difference is that in the latter the optimal frequency of stimulation is lower than in the former; the latter, therefore, only corresponds to the former when the preparation is under unfavourable condition. Another striking difference, though not a constant one, is seen to exist between direct and reflex tetanus produced by pessimal intensity of stimulation. The muscle, in the latter case, often enters into a more exaggerated contraction or goes into contraction (if this has not been present during its course) when the stimulation is discontinued. The after-discharge which appears in any reflex contraction does not usually surpass the height of tetanus obtainable during the application of stimulation, except when the reflex is produced by pessimal intensity of stimulus.

These observations on optimal intensity have a superficial resemblance to those of Sherrington and Sowton (17) on the tonic contraction of the knee-extensor which was augmented by weak stimulation and inhibited by stimuli of stronger intensity. A similar change of response to stimuli of altered intensity is exhibited by the opening muscle of the claw of the crayfish (3, 7, 13, 15).

III. *The Comparison of Reflex Tetanus with Peripheral Tetanus at varying Rates of Stimulation.*

To examine this point, the afferent nerve was faradised at the optimal intensity for a given frequency-rate, and the resulting reflex tetanus registered by a spring myograph. For comparison, a direct tetanus was produced by faradisation of the motor nerve under the same condition of stimulation.

The differences in the observations of the previous workers (Marey, Bohr, Bernstein, Kohnstamm, Wedensky, Hoffmann and others) on myal and neuromyal tetanus produced at varying rates and intensities of stimulation are to be explained by the different conditions under which the problem was studied. Some observations necessary for the present inquiry in this respect were repeated with the method which I have used in these experiments.

The extent of direct tetanus with increasing intensity of stimulation of a given frequency-rate only increases up to a certain point, and thereafter shows no further increase. The optimal intensity of stimulation for reflex excitation is usually somewhat higher than the maximal intensity for direct tetanus. A series of records of neuromyal tetanus, which was made with progressively rising frequency-rate of stimulation of maximal strength, and in which the stimulation was continued until the maximal contraction was attained in each case, shows no significant increase in the power of contraction with increase of the frequency-rate of stimulation beyond about 50 per second. From these observations it appears that the maximal tetanus produced by the maximal intensity of stimulation for a given frequency greater than 50 per second can be regarded as the maximal contraction of the muscle.

On comparing reflex tetanus with direct tetanus produced at low frequency-rates of stimulation (under 30 per second) with the same intensity, the former is usually more abrupt and more powerful than the latter in the early period of contraction. This fact shows that the discharge of impulses from the reflex centre is at a higher rate than that of stimulation applied to the afferent nerve, and further suggests that some of the component shocks used as stimuli evoke from the spinal reflex centre repetitive volleys of motor impulses, since it has already been shown that at low frequency-rates the rhythm of repetition of volley discharges from the centre is synchronous with the rate of stimulation. A similar conclusion was drawn by the comparison of reflex contraction produced by a single strong momentary stimulus applied to the afferent nerve with a direct twitch produced by a like stimulus (5, 16). If excitation is applied longer at this rate the direct tetanus increases in power, while the reflex tetanus decreases, or slowly increases, only to decrease later, so that the former always surpasses the latter in the maximal power produced. This indicates that fatigue is more easily produced in the central than in the peripheral mechanism.

At the optimal rate of stimulation the reflex contraction is often nearly as powerful and abrupt in the first period of tetanus as the direct one (fig. 4). If the excitation is prolonged, the direct tetanus soon attains the maximum, which is maintained for a long time, while the reflex tetanus diminishes in height. The difference of the maximal contraction in both cases is often extremely slight in good preparations. This observation suggests that the spinal centre is a physiological unity, any one afferent nerve being connected with all the motor neurons of the reflex arc, since there is some evidence that each component shock of faradisation applied to an afferent nerve at the rate of more than 50 per sec. is followed by only one motor impulse, not by a group of impulses (1). The result more usually obtained, that reflex tetanus falls more or less short of direct tetanus, is partly due to the fact that the reflex centre is more easily fatigued than the muscle itself. It must further be remembered that it is often difficult to make out exactly the optimal intensity. At rapid frequency of stimulation the direct is usually more powerful than the reflex tetanus.

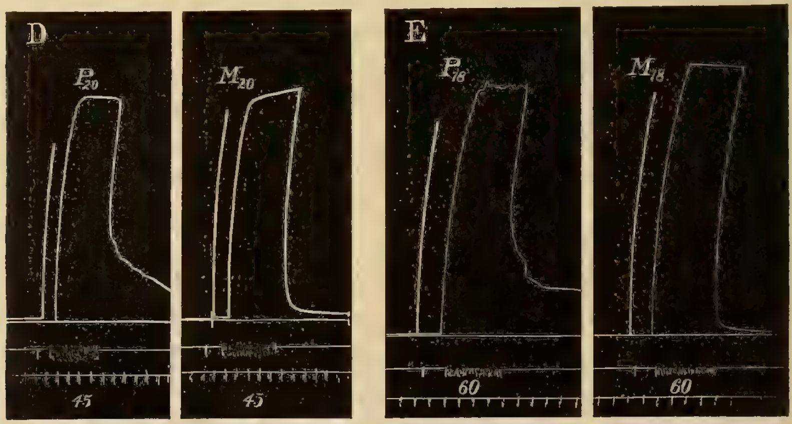

Fig. 4.—Semitendinosus preparation. Comparison of reflex (P) with maximal direct tetanus (M). D = frequency 45 per sec. and 20 c.m.; E = 60 per sec. and 18 cm.; P = peroneal nerve stimulation. M = sciatic roots stimulation. Time in sec.

The afferent nerves hitherto used were nerve trunks, such as the tibialis, peroneus, or sciatic. The extent of the reflex contraction produced by stimulating these afferent nerve trunks shows only small differences. A stronger reaction is not necessarily obtained by stimulating more afferent nerve fibres. The comparison of the maximal reflex reaction produced by exciting either a very slender afferent nerve or the skin of toes with that evoked by faradising a nerve trunk has an important bearing on the question of the physiological unity of the spinal reflex centre.

The ramus cutaneus medius was isolated with a tiny piece of the skin which it innervates. The threshold value for this nerve is usually higher than that of the peroneal trunk, a fact which may be related to the ease with which it is injured. Nevertheless, sufficiently strong faradisation of the nerve when it is freshly prepared and has a comparatively low threshold value often produces as powerful a reflex contraction as does that of the peroneal. The extent of the reflex reaction evoked by a small nerve appears to depend more upon the intensity than upon the rate of stimulation. The extent of reflex reaction can be graded with graded strength of excitation. Sometimes the skin of toes was excited by applying a pair of electrodes two or three millimetres apart. The reflex contraction thus produced often attains the intensity of that evoked by stimulating a nerve trunk (fig. 5). The course of the reflex response elicited by a stimulation of a small afferent nerve or a skin-spot is irregular.

The fact that the reflex contraction produced by a stimulation of a small afferent nerve or of a circumscribed skin point is of the same extent as that elicited from a nerve trunk and sometimes nearly equal to the maximal direct tetanus suggests strongly the physiological unity of the reflex centre, any afferent nerve, even a small skin nerve, being connected with the whole motor reflex centre.

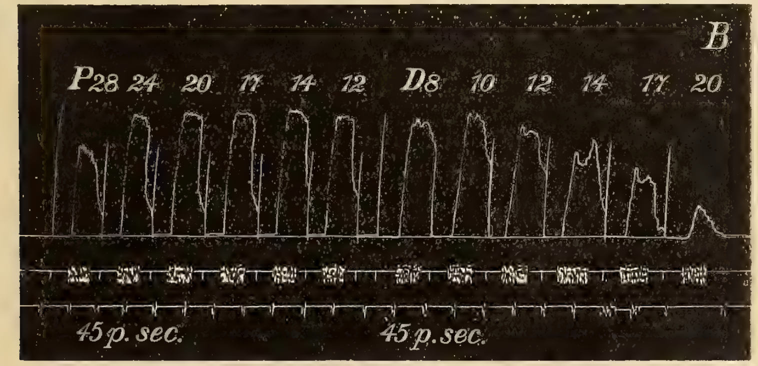

Fig. 5.—Semitendinosus reflex preparation. Responses at same frequency of stimulation (45 per second) with varying intensity. P = peroneal nerve; D = skin (2nd toe) stimulation. Numbers = distance between coils. Time in sec.

Conclusions.—(1) There is an optimal intensity of stimulation at rapid frequency-rates, *i.e.*, more than 60 per sec., for the reflex power produced in the semitendinosus muscle of the spinal frog, if an ipsilateral afferent nerve trunk is stimulated. At moderate frequency-rates (50 per second on average) the reflex result depends but little upon the intensity of excitation. At low rates (under 30 per second) the reflex response increases with the strength of stimulation, at first quickly and then slowly. The maximum, when attained, is maintained for further increments of stimulation; no optimum is usually seen.

(2) Similarly there is an optimal rate of stimulation for any given intensity which is usually about 50 per second. At the optimal intensity of stimulation for a rapid frequency the reflex power at this frequency may often be equal to or even somewhat greater than that produced at the optimal rate.

(3) The reflex myogram obtained by the resonance method exhibits mechanical vibrations synchronous with the frequency-rate of stimulation when this is under 30 per second. The spinal reflex centre therefore follows at the same rate the rhythm of successive volleys of centripetal impulses.

(4) The reflex tetanus evoked by faradising at sufficient intensity an afferent nerve at rates less than 30 per second, is more abrupt and more powerful in the first period of contraction than the direct tetanus produced under the same condition of excitation, though the latter always surpasses the former when the stimulation is prolonged. Therefore some of the component shocks used as stimuli produce a repetitive series of motor impulses from the reflex centre.

(5) The reflex contraction evoked at the optimal rate of excitation is often nearly as powerful as the direct one obtained by similar stimulation, especially in the first period of contraction.

(6) The faradisation of a very small afferent nerve-branch (ramus cutaneus medius) or of a circumscribed skin spot often produces as powerful a reflex contraction as that obtained by stimulation of a nerve trunk at the same frequency. The results obtained in (5) and (6) suggest that the motor reflex centre is a physiological unity; any afferent nerve, even a small one, being connected with all the motor neurons of a reflex arc.

I am greatly indebted to Prof. C. S. Sherrington for continuous suggestions and advice during the progress of these experiments, and for permission to utilise his instruments. I take also this opportunity in thanking Dr. C. H. Kellaway for kind assistance in expressing the results in the text.

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On the Effects of Constant Galvanic Currents upon the Mammalian Nerve-Muscle and Reflex Preparations.

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My experiments were to study the reflex effects resulting from stimulation by constant galvanic currents applied to an afferent nerve of the hind limb. The main points of enquiry have been: (1) the relation between reflex excitation and inhibition of the decerebrate tonus of the vasto-crureus; (2) whether the "excitation formula" (Pflüger's law) holds good in mammalian afferent nerves; and (3) whether there is continuous excitation during the passage of the current through an afferent nerve.

The reflex effects thus produced in the extensor muscle are complex and various, and are difficult to analyse without accurate knowledge of the results obtainable in the peripheral nerve-muscle and in the flexor reflex preparation. A few experiments were therefore made with these preparations, since the exact data required could not be obtained from the literature.

Method.—For the study of the reflex effects in an extensor muscle, the vasto-crureus of the decerebrate cat has been employed. The animal was decerebrated under deep anæsthesia with Sherrington's decerebrator. All the hind limb muscles except quadriceps were paralysed by denervation or tenotomy. The popliteal, peroneal or sciatic was used as the afferent nerve. The nerve was, though exposed previously, not actually isolated until everything was in readiness to observe the effects of stimulation, in order to avoid any changes resulting from exposure or injury to its vascular supply. The movements of the lower limb were recorded by fixing the condyle of the femur and attaching the tendo Achillis by means of a stout thread to one arm of a crank lever; the second arm, suitably weighted, functioning as a writing point. The ascent of the myogram line in the figures means contraction of the muscle; descent means inhibitory relaxation.

The constant current for stimulation was supplied usually from three accumulators. They were connected with both terminals of a rheochord, whose wire (of about 6 ohms resistance) was divided into 100 divisions. The numbers on the figures denote those of divisions of the wire short-circuited to stimulate the nerve. During the period marked by the downward notch in the signal line the constant galvanic current is applied to the nerve. Time below in seconds.

The distance between the non-polarisable electrodes upon which the nerve lay was usually 1·5 to 3 cm. and that from the ligated end to the distal electrode was more than 2 cm. The nerve was kept moist with warm saline or Ringer-Locke solution, or was sometimes enclosed in a glass tube, somewhat like the one described by Sherrington (11), in order to avoid evaporation. The direction of the constant current is described as "descending" (D, in the figures) when it flows in the afferent nerve from its cut end to the spinal cord, and as "ascending" (A, in the figures) when opposite in direction. This terminology is more reasonable in comparing the effects of constant current in the reflex and the peripheral nerve-muscle preparations, as the current in both cases passes through the nerve in the same direction with regard to the effector organ.

For the study of the reflex effects in a flexor muscle, the tibialis anticus of either the spinal or decerebrate cat has been used. The spinal transection was performed at the posterior end of the thoracic region. The nerve-muscle preparation was also tibialis anticus with its motor nerve (peroneal) isolated in the thigh. In these preparations, the distal end of the femur and tibia were fixed and the contraction of the muscle was directly recorded. Otherwise the method was similar to that already described for the extensor reflex preparation.

I.—EXPERIMENTS ON NERVE-MUSCLE PREPARATIONS.

The threshold value of stimulation for this preparation was very low as compared with that in reflex preparations. One or even half a division of the rheochord wire, through which a constant galvanic current flowed fed by one accumulator, usually suffices to provoke an appreciable contraction at closure of either descending or ascending current. With a fresh motor nerve the contraction at make generally appears with weaker ascending than descending currents. With currents of moderate strength in either direction excitation occurs both at make and break. This second stage of the "excitation formula" (Pflüger's law) appears with a rather weak current; two divisions of the rheochord are usually enough to induce opening contractions.

Very strong currents are, however, necessary to demonstrate the third stage of this formula. As a rule, I obtained no excitation at make of the ascending current, using the full potential difference obtainable from three accumulators (6 volts). It was, however, difficult to decide whether or not there was a contraction at make of the descending current, since the muscle was thrown into tetanus during its passage and appeared to relax without any further contraction when the flow of the current ceased (fig. 1). There is no doubt, however, that there is no contraction at break of a strong descending current in preparations where the motor nerve has been divided forty-eight hours before the experiment, as the muscle responds only at its make, remaining quiescent all the time the current is passing.

The height and form of the closure- and opening-contractions are different according to the strength and direction of the current. Though these contractions are not twitches in a strict sense, they are twitch-like if produced by weak currents. The contractions at make of the descending and at break of the ascending current increase with the intensity of the stimulation, while those at break of the former and at make of the latter attain their maximum, remaining on it within a considerable range, then diminish their height with further strengthening of stimulation, and finally fail to appear when the current is still stronger (fig. 1).

With weaker polarising currents there is no continuous excitation during their flow through the nerve in either direction. With progressive increase of the strength of the descending current, the make-contraction is no longer twitch-like, but is a brief tetanus, subsiding somewhat slowly. The stronger the current the more prolonged is the contraction, until such strength is reached as produces a contraction of the muscle, which lasts during the whole continuance of passage of the current. When the current is strong this closure tetanus is smooth and regular (fig. 1). During the flow of the current it declines at first quickly, then more gradually, and finally at break relaxes more or less quickly.

With the ascending current there is usually no continued tetanic contraction, whatever the strength of the current may be. A not infrequent exception to this rule is, however, that, with an ascending current of moderate strength, a weak and somewhat rapidly declining tetanus does occur during the passage of the current; but with a strong ascending current no contraction whatever occurs during the passage of the current, although on cessation of the stimulus a tetanus immediately ensues. This last seems to be a similar phenomenon to the "Ritter's opening tetanus" in the frog.

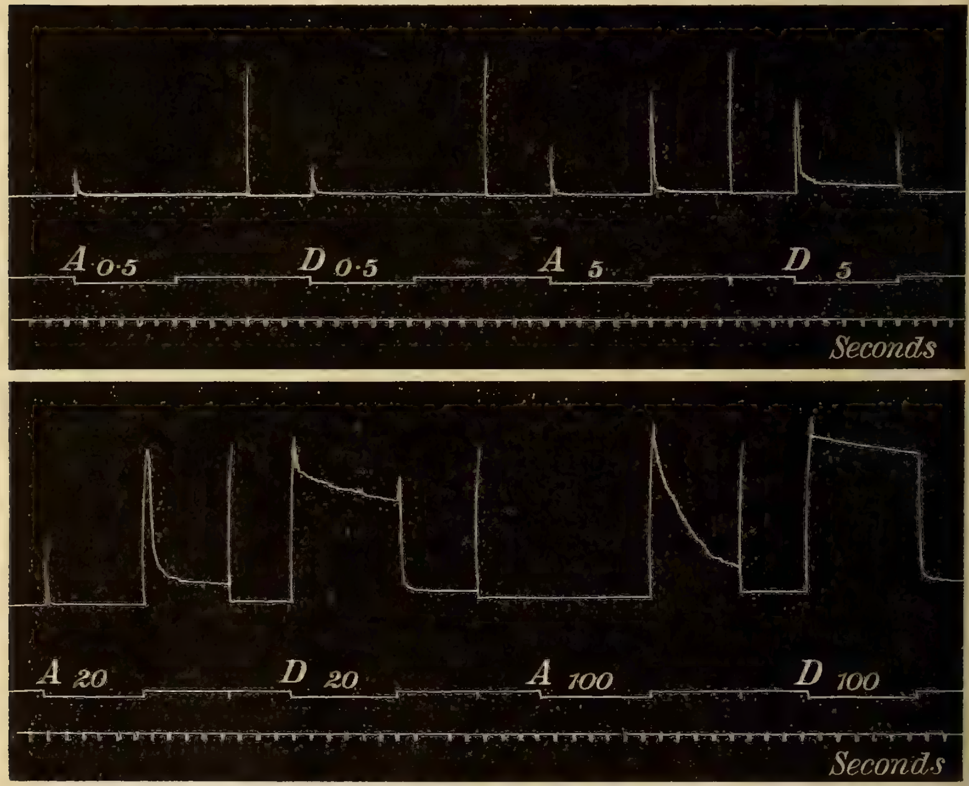

Fig. 1.—Nerve-muscle preparation of cat, showing response to constant current stimulation. A = ascending; D = descending.

The remarkable fact that continuous excitation is obtainable during the passage through a mammalian nerve of descending current of sufficient strength, while its muscle remains quiescent if the current is ascending in direction, was first observed by Eckhard (3). The difference which exists between the reactions given by the nerve-muscle preparations of cold-blooded and warm-blooded animals is partly explained by the extreme susceptibility of the isolated tissues of the latter to nutritional and temperature changes. Impairment due to these causes is progressive and depends on the period of exposure, as is shown by the following experimental observations:—(1) The threshold value of the effective stimulus increases and the height of the resultant contraction diminishes with lapse of time after isolation. (2) If the motor nerve has been exposed for some time, the break contraction is often produced with a smaller ascending current than is the make. (3) If the nerve is still more impaired, a constant ascending current causes an apparent relaxation of the muscle at make which is removed only at break (fig. 2). On the other hand, a small contraction occurs at make of the descending current, which is maintained during its flow. The damage to the nerve appears to be the cause of continuous stimulation, which induces without

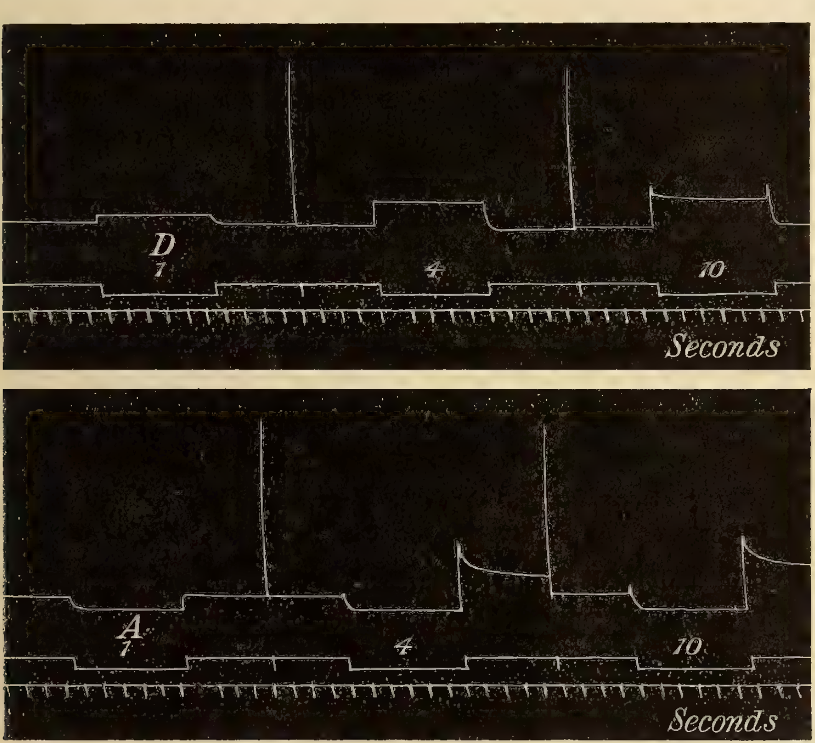

Fig. 2.—Nerve-muscle preparation of cat with the nerve left exposed after its isolation. The muscle is in a "tonic" contraction which is depressed by the ascending and augmented by the descending current.

any external stimulus, a weak tetanus, which is either augmented or depressed by the onset of catelectrotonus or anelectrotonus. It may be therefore supposed that in a fresh mammalian nerve, even immediately after isolation, subliminal stimuli due to injury are present, and when the impairment of the nerve is advanced, the muscle is already in a state of weak tetanus.

The closure and opening tetanus seen in the mammal may be explained in the same way as in the frog, if we assume that these subliminal stimuli due to injury and exposure become effective from the positive modification in excitability at the catelectrotonic tract of the nerve during the closure of the current, and at the previously anelectrotonic tract on opening the current.

The question arises here as to whether these pre-existing stimuli are due to an injury current or to other intrinsic causes connected with the dying process of an isolated tract of the nerve. To decide this, the sciatic nerve was divided twenty-four or more hours before the experiment, since the injury current diminishes greatly in that time. The results obtained in such preparations were identical with those already described. The injury current does not appear, therefore, to play an important *rôle* in this respect. When the animal is left alive more than forty-eight hours after aseptic division of the sciatic nerve, the threshold value of the nerve rises, and continuous excitation is no longer obtained during the passage of strong currents in either direction. Even in the third stage of the excitation formula, no appreciable tetanus takes place during the flow of descending current through the nerve. The nerve loses entirely its mechanical and electrical excitability if the animal is allowed to survive three days after the nerve section. This reaction of degeneration does not directly concern us here.

II. EXPERIMENTS ON THE FLEXOR REFLEX PREPARATION.

These results are on the whole similar to those obtained in the nerve-muscle preparation. The threshold value for the spinal preparation was often found to be nearly as low as that of the nerve-muscle preparation. It was, however, much higher in the decerebrate (sometimes more than ten times higher) than in the nerve-muscle preparation (Sherrington (14) and others).

Pflüger (7) himself showed that the law which he formulated for the motor nerves held for the afferent nerves of the frog. In the mammalian reflex preparation, the second and third stages are more easily demonstrated than the first. The first stage can usually be verified if the threshold value is low. When it is high, as in the decerebrate preparation, the contraction at break of the ascending current often makes its appearance at a lower value of the current stimulus than does the contraction at make, while the reverse is true of descending currents.

In some flexor preparations, both decerebrate and spinal, with freshly isolated nerves, no continuous excitation takes place during the passage of either fairly strong ascending or descending currents. More usually, however, reflex tetanus is produced at make of the current, if the current be strong and of descending direction. The intensity of current necessary to produce this result is far greater in these preparations than in the nerve-muscle preparation. Moreover, this closure reflex tetanus usually subsides some time during the course of prolonged stimulation.

With the flexor reflex preparation therefore, even when that preparation is of the spinal kind, which is more readily responsive reflexly than the decerebrate, the galvanic current even when strong evokes from the afferent nerve a contraction only at make and break, and not during the maintained passage of the current, or does so only exceptionally. This is a striking difference from the nerve-muscle preparation.

III.—EXPERIMENTS ON THE EXTENSOR REFLEX PREPARATION.

The experimental results in this preparation are more complex, and it is sometimes difficult to analyse the various factors concerned in causing this complexity. Under favourable conditions the results, though manifested in a different way, agree in regard to the effects of changes in strength and direction of current with those obtained in the nerve-muscle and flexor reflex preparations. These reactions can be more easily understood when the influence of certain characteristic features of the central nervous system is considered. Weaker or less abrupt stimulation of an ipsilateral nerve, as Sherrington and Sowton (12) showed, tends to produce contraction in the knee-extensor, while stronger or more abrupt stimulation always results in inhibitory relaxation. Constant currents are more suitable for the study of this phenomenon than induction shocks. The other important features of the central nervous mechanism which are related to the present enquiry are tonic plasticity (10) and the "rebound" phenomenon.

The general experimental results with this preparation are recorded in four sections.

(1) *The Validity oj Pflüger's Law.*

In excitable preparations, the reflex movement, whether excitatory or inhibitory, appears with smaller intensity of stimulation at make of the either descending or ascending current than at its break. If, however, the threshold value is rather high from the first the reflex effect at break of the ascending current has a threshold which is either lower than or equal to that at its make (see above). Even in this case it is always greater at make of the descending current than at its break. This result agrees with that obtained in the flexor reflex preparation. It corresponds to a stage of Pflüger's law

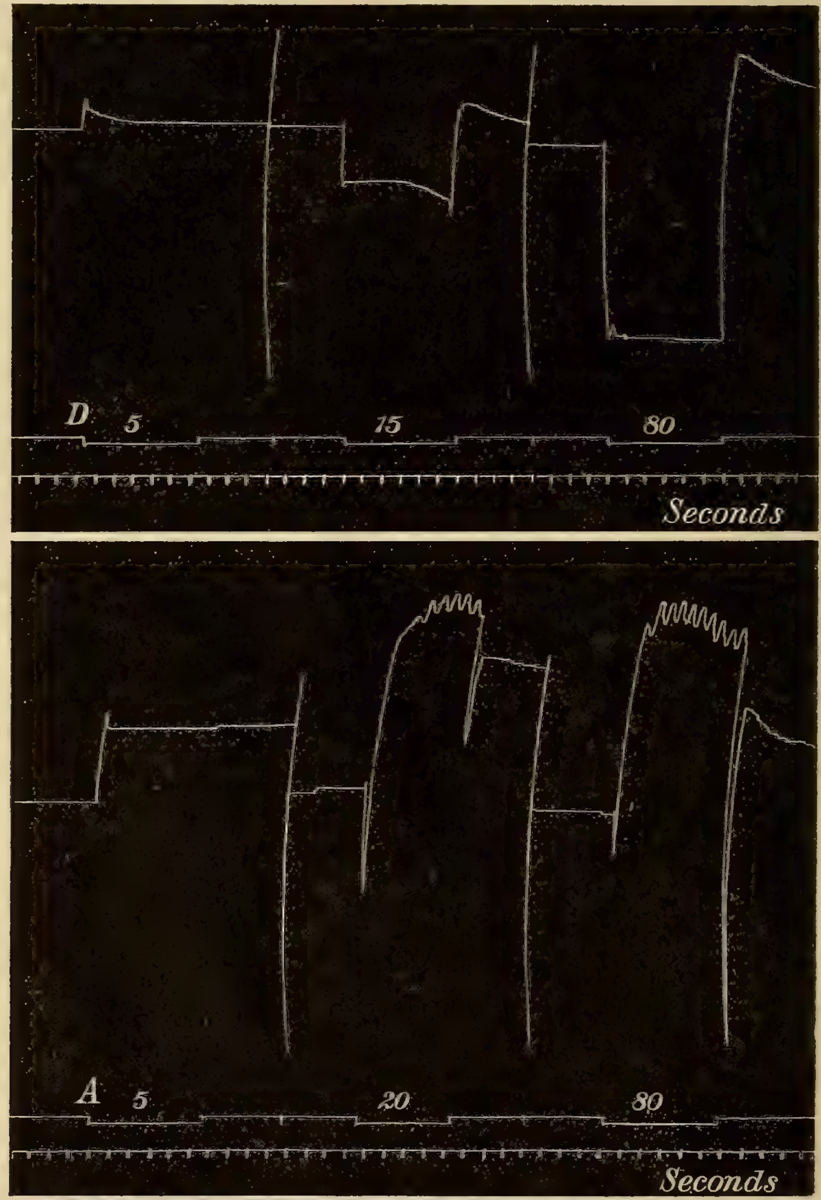

Fig. 3.—Extensor reflex preparation of decerebrate cat, showing response to constant current stimulation, applied to the proximal stump of the cut sciatic nerve.

which in the nerve-muscle preparation is reached only after the current has been made stronger than that which gives the initial stage of the law. The second stage of the law of polar excitation is attained by doubling the intensity of the current and is still elicited within a considerable range. By further intensifying the stimulation, the reflex effect produced by closing the descending, and by opening the ascending current, increases progressively to a maximum (fig. 3).

It is often difficult to make out the relation between the intensity of the current and the extent of the reflex effects produced at break of the descending, and also, though in a less degree, at make of the ascending current, since many factors are involved in the reaction. Under favourable conditions of experiment, as in the preparation where any reflex effect is only inhibitory, these reflex effects sooner or later attain a maximum at a certain optimal strength of current, and then gradually diminish in extent with further increase of strength of the current, so that the third stage of Pflüger's law holds good in the extensor reflex preparations.

(2) *The Relation between Reflex Excitation and Inhibition.*

In preparations of good decerebrate rigidity, excitation is usually produced by a weak current, both at make and at break, in either direction. The myogram line rises abruptly and either drops down again more or less quickly to the initial line or remains at the new position. When this shortening reaction following an active reflex contraction is well pronounced, and the stimuli at both make and break take effect, the myogram exhibits two steps. The excitatory reflex effect increases with the strength of current within somewhat narrow limits. As the current is increased, the reflex contractive effects produced at make of the descending and at break of the ascending current, first of all diminish in extent, and finally pass over into inhibitory relaxation which becomes progressively greater with further strengthening of the stimulus.

Similarly, at make of the ascending and at break of the descending current reflex contraction gradually changes into inhibitory relaxation when the strength of the current is step by step increased. The relaxation in this case is always limited. In some preparations where the decerebrate rigidity is well marked, no relaxation of the muscle is induced at all, and only excitatory effects are obtained, whatever the strength of the current employed. This result may be explained by assuming that, as the stimuli at make of the ascending and at break of the descending current do not exceed a certain intensity, such relatively weak stimuli in such preparations cause only excitation.

(3) *On Continuous Excitation in the Nerve during the Passage of the Current.*

During the flow of weak constant galvanic currents in either direction, no continuous reflex effect occurs. With stronger stimulation, the reflex phenomena obtainable during the passage of current through the nerve are different according to its direction, as was seen by Sherrington and Sowton (12). With the ascending current, the excitatory effect at its make increases progressively with the strength of excitation and then often passes over into a rebound contraction following an inhibitory relaxation of short duration. The rebound contraction is, in its turn, followed by a shortening reaction or relaxes gradually. A passive plastic reaction is easily obtained during the passage of strong ascending currents, except immediately after its closure when the rebound phenomenon is still in progress. This is a convincing proof that no excitation is occurring during the passage of even a strong ascending current. This rebound phenomenon is, however, often so prolonged that one may think that excitation is still in progress. In such a case, the lengthening reaction is more limited than before the application of the current. This excitatory process is neither more nor less than a prolonged rebound phenomenon. If the preparation is of poor rigidity, where any reflex effect is only inhibitory relaxation of the tone of the muscle, the effect at make of a constant ascending current of sufficient strength is inhibition followed by a lengthening reaction without any rebound contraction. This is another sign that there is no continuous reflex effect during the passage of the current, since the plastic reaction is unchanged by its application.

At make of a stronger descending current the tonic contraction of the muscle relaxes. This relaxation is somewhat prolonged, and is followed by a rebound contraction weaker than that produced by a like stimulus of ascending direction. This seems to correspond with the result seen in the nerve-muscle preparation, that the contraction at make of currents of moderate strength is prolonged in its phase of relaxation. The poor rebound phenomenon may result from depression by a prolongation of the inhibitory effect at make, so that the resulting level of the myogram line is determined by the algebraic sum of excitatory and inhibitory processes. By further increase in the intensity of stimulation, the inhibitory relaxation becomes more complete and the succeeding rebound contraction is less marked, until the former attains its full extent and the latter no longer takes place. The phenomenon is not merely a lengthening reaction following inhibition, since the muscle is felt to be more flaccid than before the application of the current, and the passive shortening reaction is no longer easily obtainable. If the lower limb is then passively extended, so that the myogram line is at a high level, this falls slowly and steadily during the flow of a strong descending current. The strength of stimulation required to produce a continuous effect in this case is far greater than that in the case of nerve-muscle preparations. The inhibitory reflex effect at break of a powerful ascending current is often prolonged. The rebound contraction following it, is limited, as it is depressed by prolongation of the inhibitory process.

The above experimental data show that there is a continuous reflex effect during the passage of a strong descending polarising current through the afferent nerve in the extensor reflex preparation, which is not present if the current is reversed in direction. The results in this preparation of stimulation by constant galvanic currents show full agreement with those in the flexor reflex and nerve-muscle preparations. In order to produce a continuous effect from the afferent nerve, the current required is, however, far stronger than that for the nerve-muscle preparation.

These reflex results in the extensor reflex preparation bear some resemblance to the changes in the respiratory movement observed by Langendorff and Oldag (5), as a result of the application of constant galvanic currents to the central stump of the cut vagus.

In some preparations in which walking movements occurred without any artificial stimulation, these movements were inhibited whenever a weak polarising current was led into the nerve in descending direction. On the contrary, ascending currents either augmented the movements or produced no effect upon them. Sometimes, in the latter case, the walking movements, which were not present before stimulation, were superadded to the usual reflex results (fig. 3).

(4) *The Response by Excitation or Inhibition modified by Factors other than those involved in Stimulation.*

The complexity and multiplicity of reflex results in the extensor reflex preparations are dependent on other factors than those already mentioned as influencing the functional condition of the central nervous mechanism. In the presentation of the results of these experiments, as detailed above, the more typical have been selected. There were, however, cases in which the responses were altered, by variation in the degree of decerebrate rigidity, in the initial length of the tonic muscle, and in the position of the neck and other joints:—

(i) The intensity of decerebrate rigidity developed in the preparation varies in different cats and is influenced by the depth of anæsthesia and the time which elapses after decerebration. In some preparations, especially those with poor rigidity, excitatory reflex effects are entirely, or almost entirely, absent, and only inhibitory reflex effects can be obtained. No rebound phenomenon occurs even after strong stimulation. Plastic reaction is, however, fairly easily obtainable. At a certain stage of anæsthesia, a preparation showing typical reflex responses of excitation and inhibition before administration of the drug is converted to one in which the excitatory reflex effect is entirely excluded. The anæsthetic (chloroform and ether) can convert an excitatory into an inhibitory reflex effect, as was first pointed out by Bayliss (1) in the case of the vasomotor pressor reflex, and by Sherrington and Sowton (13) in the vasto-crureus reflex preparation. In other preparations, on the contrary, excitatory effects are obtained with comparatively strong currents in either direction. Only the strongest stimulation of the afferent nerve produces relaxation of the tonic contraction of the muscle, and the inhibition is always followed by a marked rebound contraction.

(ii) Another condition which influences the excitatory and inhibitory effects is the initial length of the muscle. The muscle in the lengthened condition reacts by excitation more easily to any stimulation, and in the shortened condition by relaxation. If the functional condition of the tonic centre favours the extensor tone, the reflex effect as obtained by weak stimulation of the afferent nerve is often excitation, whether the muscle be lengthened or shortened. The contraction in the former condition is always more ample than that in the latter. Conversely, if the condition of the tonic centre is unfavourable to the tone of the extensor muscle, the reflex effect is usually relaxation, irrespective of the length of the muscle, especially with strong stimuli. The inhibitory relaxation is always greater in this case, the more shortened the muscle. Between these two extremes it is often observed that reflex contraction is induced by a given stimulus when the muscle is lengthened, while the inhibition takes place with the same stimulus when it is shortened (fig. 4).

It has been noticed that the posture of the limb influences and can even reverse the direction of reflexes obtainable from the limb (6, 8, 9). But such observations have rested on experiments in which there has been no actual isolation of the muscle which responds to the reflex stimulus employed. In the present instance the observations I have obtained have been made on the actually isolated muscle.

(iii) The position of most of the other joints in the body has some influence upon the mode of reflex reaction (Beritoff (2), Magnus (6)). Above all, the position of the neck in regard to the trunk and that of the head in space must be kept constant throughout the experiment when the relation between excitatory and inhibitory reflex effects is under investigation. Reflex reversal is often obtained by twisting the neck from one side to the other (fig. 5).

The reflex reversal by altering other conditions of experiment than those here described, does not, however, directly concern the present experiments.

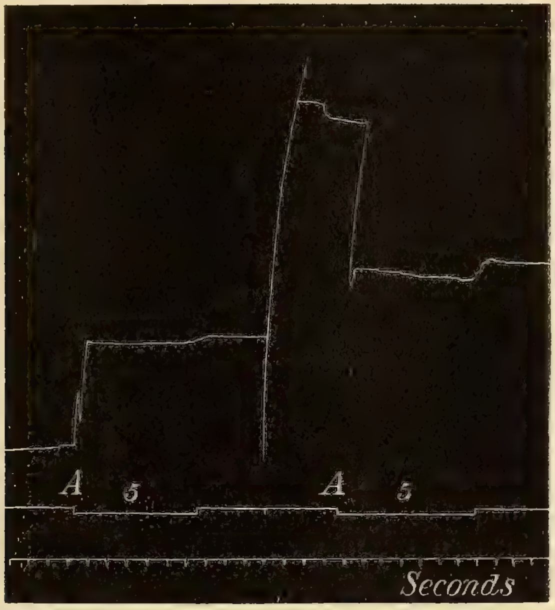

FIG. 4.—Extensor reflex preparation of decerebrate cat, showing reflex reversal by alteration of initial length of the tonic muscle.

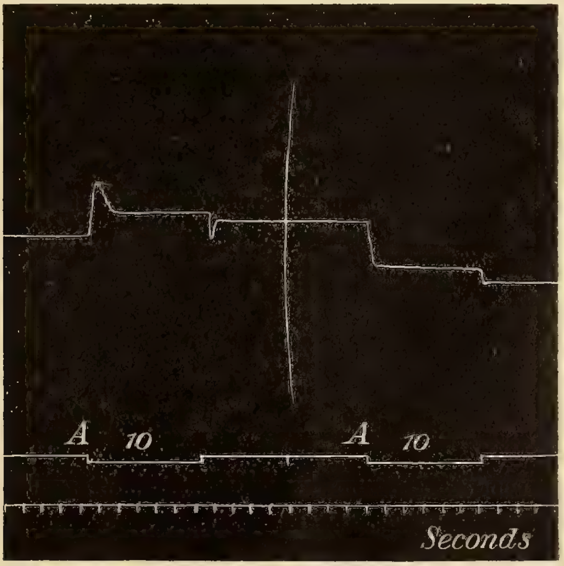

FIG. 5.—Right extensor reflex preparation of decerebrate cat, showing reflex reversa obtained by altering the posture of the head from the right to the left side.

In regard to those other conditions, the article, "Reflex-umkehr" ('Ergebnisse der Physiologie') by Graham Brown (4), where the cases are exhaustively summed up, may be consulted.

Conclusions.

(1) The "excitation formula" (Pflüger's law) holds good in mammalian nerve both efferent and afferent. When the threshold value of stimulation is high, as in some decerebrate reflex preparations, the contraction at break of the ascending current takes place in an earlier stage than that at its make, though the reverse is true if the current is descending.

(2) During the passage of a weak constant galvanic current, no continuous excitation is produced in either nerve muscle or reflex preparation. If a stronger descending current is passed through the nerve, this occurs in both preparations; though if the current is ascending, this is usually not the case. Far stronger currents are necessary to produce it in the reflex than in the nerve-muscle preparation.

Two days after division of the sciatic nerve, no continuous excitation is produced during the passage through the nerve of constant current, however strong, either ascending or descending.

(3) In nerve-muscle preparations, if the nerve is in bad condition after prolonged exposure, it acquires the character of responding more easily, with tetanus at the make of the descending and at break of the ascending current. When the condition is advanced the muscle seems to be in a weak contraction even when no stimulus by the current is being given to it: the passage through it of an ascending current then causes a relaxation of the muscle during the passing of the current.

(4) As to the relation between reflex excitation and reflex inhibition the following can be stated:—

The intensity of the inhibitory and excitatory reflex effects on the tonus of the vasto-crureus muscle and the relation between these two effects are determined by the strength, duration and direction of the stimulating current, but are also influenced by the degree of tonus in the preparation, the initial length of the muscle, and the position of the neck and other joints. Any condition which favours the tonus of the extensor muscle causes increase in the excitatory tendency of the reflex effect and diminution in the inhibitory tendency, and *vice versâ*. The reversal of reflex effect is thus often obtained by altering these conditions without changing those of stimulation.

I am greatly indebted to Prof. C. S. Sherrington for suggestions and advice during the course of these experiments.

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On the Longevity of certain Species of Yeast.

By Arthur R. Ling and Dinshaw Rattonji Nanji.

(From the Department of Biochemistry of Fermentation, University of Birmingham.)

(Communicated by Sir Edward Thorpe, F.R.S. Received July 7, 1921.)

In the autumn of 1918 Dr. J. J. Hood handed to one of us (A. R. L.) eight cultures of yeast, belonging to the late Mr. A. Gordon Salamon. The cultures in question, it appears, were given to Mr. Salamon by the late Prof. Hansen in 1887. They consisted of Freudenreich flasks, containing wads of perfectly dry cotton-wool. The flasks were furnished with a side tube and there was a tube at the apex of each hollow stopper. There was a cotton-wool plug at the opening of each flask inside the hollow stopper, and the tube at the apex of the stopper was plugged with cotton-wool, as was also the side tube. In addition to this, the cotton-wool plug of the side tube was coated with sealing-wax and the hollow stopper was also fixed on with a ring of sealing-wax. Each flask bore a label, giving the name of the particular yeast and the date in Prof. Hansen's handwriting. The following is a copy of the script on each label:—

Sacch. Pastorianus I, Hansen, 17/9/87.
Sacch. Pastorianus II, Hansen, 17/9/87.
Sacch. Pastorianus III, Hansen, 17/9/87
Sacch. ellipsoideus I, Hansen, 17/9/87.
Sacch. ellipsoideus II, Hansen, 17/9/87.
Sacch. exiguus, 17/9/87.
Sacch. cerevisiæ I, Hansen, 17/9/87.
Carlsberg Unterhefe, No. 2. 17/9/87.

As already observed, the contents of each flask consisted of a dry cotton-wool plug and the plugs were in every case of a brownish colour. Apparently, the cultures had originally been made in the manner described by Prof. Hansen in his 'Studies in Fermentation,' 1896, p. 74, thus:—

"For sending pure cultivated yeast in an absolutely pure condition, and in such a manner that its multiplication can be effected easily and with certainty, I have likewise made use of the following method, which has also given good results:—To the small cylindrical flasks generally known as Freudenreich flasks I have added a side tube (see fig.). The tube, A, on the hood is, as usual, filled with cotton-wool; a firm layer of cotton-wool is placed at the bottom, E, of the flask, and a plug is inserted in the neck, B. The side tube is also plugged with cotton-wool, and the flask is then sterilised by heating it for two hours at 150° C. When it has cooled, the tube is joined to the rubber of a two-necked flask, in which the yeast has been grown, and a drop of the fairly thick yeast is poured on to the layer of cotton-wool, E. The tube is then closed by a stopper, D, of asbestos card, previously sterilised in a flame, and the stopper is then coated over with a layer of sealing-wax, C."

In order to ascertain if the yeasts were still alive, a little sterile sweet wort was introduced into each flask by the side tube after removing the wax and cotton-wool. The flasks were then kept in an incubator at 25° until growth appeared. A thick sediment formed after some time, and when this was examined it was found in every case to contain yeasts, sometimes contaminated with bacteria. Plate cultures were made on wort gelatin containing 0·4 per cent. of tartaric acid, and ultimately pure cultures of the several yeasts were obtained.

The cultures were identified by Hansen's spore method, the results being shown in the following Table:—

Hansen's Yeasts.

Species.	Time of first appearance of spores observed in a fresh 24 hours' growth at 25° C.	Size of the spores.
Sacch. cerevisiæ	Within 24 hours	2½–6μ
Sacch. ellipsoideus I	Within 24 hours	2 –4μ
Sacch. ellipsoideus II	Within 36 hours	2 –4μ
Sacch. Pastorianus I	Within 36 hours	1½–4μ
Sacch. Pastorianus II	Within 24 hours	2 –4μ
Sacch. Pastorianus III	Within 36 hours	2 –3½μ
Sacch. exiguus	Scanty spores after 36 hours	2 –4½μ
Carlsberg No. 2	Within 36 hours	3 –6μ

We may mention that four species of these yeasts showed a well-marked liquefying power towards gelatin, liquefaction occurring in from 2½ to 4 weeks after making the streaks and keeping at the ordinary temperature. The liquefying power occurred in the order shown below:—

1. Sacch. Pastorianus I.
2. Sacch. ellipsoideus I.
3. Sacch. cerevisiæ.
4. Sacch. Carlsberg, No. 2.

The other species did not liquefy gelatin, but here it was observed that there was a characteristic tendency for growth to extend into the depth of the gelatin culture medium.

The fact that after thirty-four years these yeasts were still living is, we think, a point of great scientific interest and importance. In what form they retained their vitality, however—whether as resting cells or spores—cannot, unfortunately, be determined. From the work of Hansen we know that one species at least, *S. apiculatus*, found on the exterior of certain fruits, hibernates in the soil, and inasmuch as this species, or at all events the yeast with which Hansen worked, does not form endo-spores, it may be that our yeasts have been preserved as resting cells.

The only observations at all parallel with our own are those of H. Will,* who showed that certain wild yeasts, on a dry asbestos medium, were still living after seventeen years. They apparently had retained the cell form.

Further experiments with these and other species of yeasts are in progress in these laboratories.

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* 'Zeitschrift für das gesammte Brauwesen,' 1896–1904.

On the Origin and Destiny of Cholesterol in the Animal Organism.
Part XII.—*On the Excretion of Sterols in Man.*

By John Addyman Gardner and Francis William Fox (Beit Memorial Fellow).

(Communicated by Prof. A. D. Waller, F.R.S. Received July 11, 1921.)

(Report to the Medical Research Committee. From the Physiological Laboratory, University of London, South Kensington.)

In Part X of this series [vol. 86, p. 13 (1912)]—"On the Excretion of Cholesterol by Man"—Ellis and Gardner, from analyses of the dried fæces collected during a series of experiments, carried out by R. H. A. Plimmer, M. Dick, and E. C. Lieb, at the Institute of Physiology, University College, and published under the title of "A Metabolism Experiment, with Special Reference to Uric Acid," came to the conclusion that in man the excretion of cholesterol in the fæces can be largely accounted for by that taken in with the food, provided that the body weight remains constant; if, however, a rapid loss in weight takes place, as in illness, the output of sterol exceeds the intake.

Further work has shown that this conclusion requires modification. In the above-mentioned investigation only one subject was experimented on and the cholesterol-content of the diet was not obtained by analysis of samples of the food actually consumed by the subject under experiment, since the examination of the fæces in question was not undertaken until long after the completion of Plimmer, Dick, and Lieb's investigation, and was of the nature of an afterthought.

The cholesterol ingested was estimated, partly from analyses of similar foods purchased long afterwards, and partly from published analyses of other observers. Further, in the examination of the fæces it was assumed that the only sterol present in the unsaponifiable matter was the crystalline coprosterol. This was separated from the accompanying oils, as far as possible, by crystallisation from alcohol or acetone. The last traces remaining in solution were obtained by conversion into the benzoate, which is only soluble with difficultly in cold alcohol. This method necessitates the use of large quantities of material to obtain accurate results.

Since the publication of this work, new and improved methods for the estimation of sterols in tissues, etc., have been introduced, and, further, our knowledge of the composition of the unsaponifiable matter of human fæces has been considerably extended.

It has been shown by one of us (1921) that though the crystalline sterols of human adult fæces consist mainly of coprosterol, there are also present smaller quantities of β-cholestanol, cholesterol, and possibly phytosterol, derived from the vegetable food. These substances can be quantitatively precipitated together from alcohol solution in the form of their digitonides, by means of an alcoholic solution of digitonin, and so estimated.

There is, however, no simple method by which the quantitative composition of the mixture of sterols recovered from the mixed digitonides can be determined. If a sufficient quantity of material is available, the bulk of the coprosterol may be separated by fractional crystallisation. The β-cholestanol may be separated from the coprosterol by conversion of the latter into ψ-coprosterol, which does not form a compound with digitonin, and the isolation of the unchanged β-cholestanol in the form of its digitonide. Cholesterol (with phytosterol) may be approximately estimated as dibromide.

These crystalline sterols, precipitable by digitonin, constitute only a proportion of the unsaponifiable matter of fæces, the rest may be washed away by means of ether or light petroleum from the precipitated digitonides and obtained as a reddish brown oil. This oil can be distilled, without any decomposition, in superheated steam and passes over into the condenser in the form of a solid emulsion, which is forced from the condenser, partly by the pressure of steam and partly by the action of the condensed water, in the form of solid white candles. These persist for a long time, but usually separate on long standing into oil and water. This oil can be distilled in a vacuum of 1 mm. without decomposition and passes over in a series of fractions ranging from 100° to 220°. The lower fractions contain aliphatic alcohols of high molecular weight, of which cetyl alcohol has been definitely identified. These fractions do not give any colour reaction with acetic anhydride and sulphuric acid. The higher fractions, boiling 200°—220° under 1 mm., constitute the main bulk of the oil. They are usually obtained in the form of transparent yellow glass, melting at 16°—18° C. and showing in bulk a greenish fluorescence. These glassy substances are marked by great stability, contain oxygen in a not very reactive form, and have a molecular composition of much the same order as cholesterol (Gardner, 1921).

They give the Burchardt-Liebermann reaction with acetic anhydride and sulphuric acid in a well-marked, though modified, manner, and though no crystalline esters have been prepared, they appear to be alcohols of a polycyclic type.

In view of the above facts, it seemed to us desirable to investigate more fully the intake and output of sterols in the case of normal human subjects on a rigidly known diet. For this purpose we made use of some of the material obtained by a sub-committee of the Royal Society Food (War) Committee in experiments on the digestibility of breads made from different kinds of flour (1918), and by Gardner and Fox in their experiments on the digestibility of cocoa butter (1919).

The Diets consumed.

The daily diet of the subjects of the bread experiments consisted approximately of—

	grm.
Bread	800–1000
Minced meat	50
Butter	50
Jam	100
Milk	600
Cheese	50
Sugar	30
Tea	*ad. lib.*

The diet of the subjects of the cocoa butter experiments was the same, except that the butter was replaced by cocoa butter.

In the Tables in this paper the diets are referred to as A, B, C, D. In A the bread was made from 80 per cent. milled flour, in B from 90 per cent. milled flour, and in C from 80 per cent. milled flour mixed with 20 per cent. maize. In D the bread used was the ordinary white bread of the shops (July, 1918). Some latitude was allowed for individual taste; for instance, subject F. J. in the bread experiments omitted the meat ration, and W. J. omitted the cheese, and subject C. took a certain amount of light beer.

Subjects used in the Experiments.

An account of the eight subjects, four in Cambridge and four in London who took part in the bread digestibility experiments is given in the above-mentioned report, and of the three subjects of the cocoa butter experiments in Messrs. Gardner and Fox's paper. The latter paper also contains an account of the fat utilisation in both series.

Experimental Methods.

The general procedure was fully described in the Royal Society report, but we may mention that the diets were consumed for periods of ten days, the six significant days being the fourth to ninth inclusive. The collection of fæces began one day later, and continued one day longer than the days of the diet. Accurate accounts of the weights of the food eaten were kept, and analyses of samples of each food consumed by each set of subjects were made, no figures published in the literature being made use of. This was very necessary, as there was reason to suppose that war-time conditions of under-feeding might have an effect on the composition of various articles of the diet, particularly milk and meat.

Methods of Analysis.

The fæces were analysed in the following manner. A weighed portion of the dried fæces was subjected to a prolonged extraction in a Soxhlet apparatus with ether, and the ethereal solution was made up to known volume. Aliquot portions were then respectively titrated with standard alcoholic caustic soda, and evaporated to dryness and weighed. The rest of the ethereal solution was then mixed with a hot alcoholic solution of a very large excess of sodium, and allowed to stand forty-eight hours. The precipitated soaps were then filtered on the pump and thoroughly washed with ether. The ethereal filtrates and washings were then repeatedly shaken with alkaline water, and finally with distilled water, until quite free from soap. The ethereal solution of unsaponifiable matter thus obtained was made to known volume, and a suitable aliquot portion evaporated to dryness and weighed. The weighed unsaponifiable matter was then dissolved in alcohol, and the boiling solution mixed with a hot 1 per cent. alcoholic solution of digitonin, using at least a 10 per cent. excess, and allowed to stand overnight for the insoluble sterol-digitonides to separate. The alcohol was then evaporated at the lowest convenient temperature, and the residue washed by decantation with ether or light petroleum, to separate the portion of the unsaponifiable matter not precipitated by digitonin. The ether washings were passed through a weighed Gooch crucible, to guard against any loss of sterol-digitonide. The mixture of digitonide and excess of digitonin was then freed from the latter by washing by decantation with warm water, and finally the sterol-digitonide was brought into the Gooch crucible and the washing completed. The digitonide was then dried at 110° and weighed.

It was found advantageous to cover the asbestos mat of the crucible with a layer of pure sand. This prevented to some extent the sterol-digitonide from forming an impervious cake on the surface of the asbestos, and thus facilitated the filtration and washing. It was also very desirable to evaporate the alcohol before washing the digitonides, owing to the fact that the digitonides of both coprosterol and β-cholestanol are considerably more soluble in alcohol than cholesterol-digitonide.

The weight of sterol-digitonide, × 0·234, equals the weight of sterol in the unsaponifiable matter taken. The sterol-digitonide, precipitated from fæces, consisted mainly of coprosterol digitonide with smaller portions of β-cholestanol digitonide, cholesterol digitonide, and perhaps also phytosterol digitonide.

The unsaponifiable matter not precipitated by digitonin was got by difference, but this was always checked by direct weighing of the oil washed away from the digitonide by ether. Fat in the foods was determined by extraction with ether in the usual way, and the unsaponifiable matter and sterols estimated as described in the case of fæces. In the case of bread and meat, however, the ether extraction was preceded by repeated extraction with boiling alcohol, as it is well known that in such substances the extraction of fat by ether alone is imperfect, even though the exhaustion may be prolonged. The alcohol extracts were then evaporated, taken up in ether, and the ether solution was added to the other ether extract.

Full details of the nitrogen balance in the subjects of diets A, B, C and D are given in the reports and papers mentioned.

Results.

The results of our experiments on the intake and output of unsaponifiable matter are summarised in the following Tables. Table I contains the daily intake and output of sterols precipitated by digitonin. The figures represent the daily average over a period of six days. The intake consists of the total cholesterol, in free and ester form, of the food consumed, together with traces of phytosterol. The sterol excreted consisted, as stated above, mainly of coprosterol, with smaller quantities of β-cholestanol and cholesterol, and perhaps phytosterol. The subjects are indicated by their initials. Eight of the subjects partook of diets A, B and C, except Mr. C., who was omitted from the experiment on diet B owing to an attack of diarrhœa, which came on during the experimental period.

Only two of these subjects, E and B, were available for the experiments with diet D. Another subject, P, joined in this experiment.

In Table II, the daily intake and output of the portion of the unsaponifiable matter of the fat which is not precipitated by digitonin is given. These figures are only approximate, and are no doubt slightly too high owing to the presence of traces of resinous matter produced by the action of the alkali on the alcohol during hydrolysis of the fat (Gardner and Fox, 1921).

Discussion of Results.

It will be noticed from the figures in Table I that in every case there is an excess of output over intake, except subject B on diet C, who shows a

Table I.—Daily Intake and Output of Sterols precipitable by Digitonin in grammes.

	E.	C.	B.	A.C.	H.W.H.	F.J.	W.J.	A.L.	
Diet A—									
Intake of sterol in food	0·28	0·28	0·30	0·22	0·26	0·15	0·19	0·23	
Output of sterol in fæces ...	0·67	0·54	0·56	0·45	1·07	0·28	0·65	0·31	
Balance	−0·39	−0·26	−0·26	−0·23	−0·81	−0·13	−0·46	−0·08	Average loss of sterol −0·34.
Diet B—									
Intake of sterol in food	0·25	—	0·26	0·23	0·27	0·18	0·25	0·25	
Output of sterol in fæces ...	0·59	—	0·29	0·70	0·51	0·84	0·71	1·20	
Balance	−0·34	—	−0·03	−0·47	−0·24	−0·66	−0·46	−0·95	Average loss of sterol −0·46.
Diet C—									
Intake of sterol in food	0·28	0·27	0·33	0·28	0·40	0·22	0·34	0·30	
Output of sterol in fæces ...	0·79	0·43	0·28	0·55	0·60	0·44	0·60	0·39	
Balance	−0·51	−0·16	+0·05	−0·27	−0·20	−0·22	−0·26	−0·09	Average loss of sterol −0·21.
Diet D—		P.							
Intake of sterol in food	0·19	0·19	0·19						
Output of sterol in fæces ...	0·35	0·27	0·50						
Balance	−0·16	−0·08	−0·31	—	—	—	—	—	Average loss of sterol −0·18.

Average of all subjects :—

Intake	0·2534
Output	0·5604
Balance	−0·3070

Table II.—Daily Intake and Output of "Unsaponifiable Matter" not precipitated by Digitonin in grammes.

	E.	C.	B.	A.C.	H.W.H.	F.J.	W.J.	A.L.	
Diet A—									
Intake in food	2 ·03	2 ·04	2 ·10	1 ·84	3 ·02	2 ·23	2 ·31	2 ·83	
Output in fæces	0 ·40	0 ·36	0 ·40	0 ·32	0 ·53	0 ·21	0 ·24	0 ·15	
Difference	1 ·63	1 ·68	1 ·70	1 ·52	2 ·49	2 ·02	2 ·07	2 ·68	
Percentage utilisation	80 ·30	82 ·34	80 ·95	82 ·69	82 ·45	90 ·58	89 ·61	94 ·70	Average utilisation 85 ·45.
Diet B—									
Intake in food	2 ·42	—	2 ·67	2 ·47	3 ·22	2 ·63	2 ·41	3 ·06	
Output in fæces	0 ·36	—	0 ·25	0 ·17	0 ·41	0 ·65	0 ·39	0 ·40	
Difference	2 ·06	—	2 ·42	2 ·30	2 ·81	1 ·98	2 ·02	2 ·66	
Percentage utilisation	85 ·12	—	90 ·63	93 ·11	87 ·27	75 ·28	83 ·81	86 ·92	Average utilisation 86 ·02.
Diet C—									
Intake in food	2 ·08	2 ·04	2 ·22	1 ·99	2 ·95	2 ·34	2 ·12	2 ·82	
Output in fæces	0 ·21	0 ·46	0 ·65	0 ·43	0 ·44	0 ·47	0 ·70	0 ·45	
Difference	1 ·87	1 ·58	1 ·57	1 ·56	2 ·51	1 ·87	1 ·42	2 ·37	
Percentage utilisation	89 ·90	77 ·45	70 ·72	78 ·39	85 ·08	79 ·91	66 ·98	84 ·04	Average utilisation 79 ·06.
Diet D—		P.							
Intake in food	1 ·04	1 ·09	1 ·14						
Output in fæces	0 ·35	0 ·16	0 ·27						
Difference	0 ·69	0 ·93	0 ·87						
Percentage utilisation	66 ·34	85 ·32	76 ·31	—	—	—	—	—	Average utilisation 75 ·99.

Average intake by food	2 ·197
Average output in fæces	0 ·378
Balance	+1 ·819
Percentage utilisation	82 ·79

small positive balance. The negative balances are very variable, ranging from 0·03 to 0·95 grm. per day. The average negative balance on diet A was 0·34, on diet B 0·46, on diet C 0·21, and on diet D 0·18. The high output on diet B, with coarse bread, may possibly be accounted for by the somewhat laxative effect of the brown bread on some of the subjects—subject C, for instance, was so relaxed that he was obliged to retire from the experiment. He stated that brown bread always had this effect on him. The average negative balance in the twenty-six experiments was 0·307. On comparing the same individuals on different diets, a similar great variation will be noticed.

The sterol excreted in the fæces is derived partly from the food and partly from the bile. The determination of the amount of bile excreted per day by a human being is a very difficult problem, and as yet reliable data are wanting. The amount is given in the text-books as from 500 to 1000 c.c. per day. The figures are derived mainly from the study of patients with biliary fistulæ. We may quote a case of a woman with a biliary fistula studied in some detail by Pfaff and Balch at the Massachusetts General Hospital (1897). They found that the total bile excreted per day was 525 grm., but the amounts excreted at different periods of the day were very variable. Such figures must, however, be taken with great reserve as an index of the amount of bile produced in the normal subject. It is known that, if bile is passed into the intestine, the secretion is increased both in concentration and in amount, and that fistula bile differs from bladder bile both in concentration and even in composition. Thus, Pfaff and Balch's bile contained only some 3 per cent. of total solid matter, while various observers have given the solid content of bladder bile as 10 to 20 per cent. Further, the percentages of inorganic constituents in fistula and bladder bile are of quite different order. However, there appears to be a general consensus of physiological opinion that in health, and when bile finds its way into the intestine, the excretion is probably larger (rather than smaller) than in the fistula cases.

Very variable values have been recorded in the literature for the cholesterol-content of bladder bile, and many of the figures must be accepted with considerable reserve, particularly those obtained before the development of modern methods of estimation of sterols. We know that cholesterol is very soluble in bile, and Moore and Roaf have shown that ordinary bladder bile is able to dissolve a good deal more than is ordinarily found. The cholesterol of the bile is mainly found in the non-ester condition. In more recent years, Peirce (1912) has examined the cholesterol-content of the gall-bladder bile in a variety of conditions by the digitonin method. The results are variable, but he regards the normal figure as about

0·15 to 0·16 per cent. If we take 550 c.c. as the amount of bile secreted per day and the cholesterol-content as 0·16, each of the subjects of our experiment should have passed into the intestine about 0·9 grm. per day, more or less, in bile solution. It is evident, therefore, that the subjects have reabsorbed a considerable amount of the cholesterol along with the bile salts in the intestine.

It has been shown in earlier papers of this series that in the case of herbivora (1912, 2), and also carnivora such as dog and cat (1913), that cholesterol given with the food appears in the blood, the cholesterol having been absorbed in the intestine with the bile salts. This is more difficult to demonstrate in the human subject, but Widal, Weill and Laudat (1912) have shown that heavy fat meals produce hypercholesterinemia, though this state is transitory. The adult human subject is marked off from other animals by the fact that the cholesterol passed into the intestine undergoes reduction at some stage to coprosterol and β-cholestanol, probably by bacterial action, though a small quantity of cholesterol escapes this process. It would seem a probable assumption that this reduction limits the reabsorption of the cholesterol. This has not been definitely proved, though feeding experiments are in progress with herbivorous animals to gain evidence on this point.

We think, however, that the considerations detailed above fully explain the very variable negative balances recorded in Table I.

It also follows that since cholesterol is an integral constituent of all cells of the body, and there is an excess of output over intake, *there must be some organ in the body capable of synthesising cholesterol.* This question we are at present investigating.

It will be seen from Table II that the intake of unsaponifiable matter not precipitated by digitonin is very much larger than the output, and that, as an average of the twenty-six experiments, there is a percentage utilisation of 82–83 per cent. The ratio of the average amount of fæcal sterols precipitable by digitonin to unsaponifiable matter not so precipitated was 1 : 0·25 to 1 : 1·18, but in the majority of cases the individual ratios are not far from the mean value.

As already mentioned, the fæcal unsaponifiable matter not precipitated was volatile in superheated steam without appreciable decomposition; thus 9 grm. only left in the distilling flask 0·14 grm. of carbonaceous matter.

On fractionation of the oil from the whole of the experiments under a pressure of 1 mm., the ratio of the lower boiling portions which did not give the Burchardt-Liebermann reaction to the high boiling "sterol" portion which did was about 1 : 4.

In the paper already referred to ("On the Composition of the Unsaponifiable Matter of the Ether Extract of Human Fæces") Gardner suggested two sources for these non-precipitable oils: (1) the substances which accompany cholesterol in the unsaponifiable matter of tissue fat; (2) the bile acids or their derivatives. With regard to the first source, it is clear from the figures in Table II that the quantity taken in with the food would fully account for that in the fæces. This, however, cannot be decided until the unsaponifiable matter of the tissue fats has been thoroughly investigated and compared with that of the fæces. With regard to the second source, probably oxidisation experiments will throw light on this. Preliminary experiments, however, proved inconclusive.

Work is being continued in both these directions, and we hope to have the honour of communicating the results at some future time.

We take this opportunity of thanking the Grant Committee of the Royal Society for help in defraying the expenses of this work.

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A Quantitative Analysis of the Growth of Helianthus annuus. Part I.—*The Respiration of the Plant and of its Parts throughout the Life Cycle.*

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1. *Introduction.*

By means of a quantitative analysis of plant growth we attempt to apportion the external and internal influences that determine the course of a plant's development. The former can be analysed as various recognised environmental factors, the latter at present may be grouped together as the "internal" factor for growth. The general methods formulated for such an analysis have been outlined in a previous paper (8). In the present series of papers, of which this is the first, an account is given of such an analysis of the growth of *Helianthus annuus.* The present paper is mainly confined to an account of the results obtained in an experimental study of the respiration of *Helianthus annuus* throughout its life cycle. As far as the authors are aware such a study of the respiration of a plant throughout its life cycle has not before been carried out.

The method has been to determine at frequent intervals throughout the life cycle, the respiration at a given constant temperature of a representative plant of a crop. From these results it is possible to estimate the respiration of a mean plant of the crop at the recorded fluctuating temperature of the field and so to obtain a measure of the rate of loss in dry-weight of the plant under field conditions. In addition, the results afford information as to the effect of age upon respiration. Furthermore, it is thus possible to compare the effect of progressing age upon the rate of respiration with the effect of age upon the relative rate of increase in dry-weight.

For each plant process or group of processes, such as respiration or growth, there is a group of factors within the plant which will affect the rate of the particular process or group of processes. Each group we will call the "internal" factor for that process, it being clearly understood that groups may have factors in common or may even be identical. This quantitative study of plant growth may enable one to elucidate the interconnection of these "internal" factors.

2. *The Respiratory Index as a Measure of the Effect of the "Internal" Factor upon the Rate of Respiration.*

In any attempt to determine the effect of the "internal" factor upon the rate of respiration throughout the life cycle of the plant the other factors affecting respiration should be standardised, that is, kept constant or not limiting throughout the experiments.

In view of our present knowledge of respiration the factors which may affect the rate of respiration per unit of dry-weight* can be classified as follows:—

1. Concentration of respirable material.
2. Effective amount of respiring cell-matter per unit of dry-weight. This we term the "internal" factor for respiration.
3. Concentration of oxygen.
4. Temperature.

Our measurements of respiration have been made per gramme dry-weight. In order to make the results strictly comparable from the point of view of the "internal" factor, the respiration should be measured when the amount of respirable material is in excess, the internal concentration of oxygen constant and the temperature at a definite constant value. Under such conditions the respiration per gramme dry-weight will be a measure, for purposes of comparison from time to time, of the effective amount of respiring cell-matter per gramme dry-weight, that is to say, of the "internal" factor.

It has been generally assumed that the concentration of oxygen in the atmosphere (*i.e.*, about 21 per cent.) provides an excess of oxygen for respiration. Recent work in this laboratory has shown that such is not the case, but that the carbon dioxide output,* starting from low pressures of oxygen in the atmosphere, increases with rising partial pressures, at first rapidly and then more and more slowly until a maximum is reached, and then decreases. The position of this maximum will presumably depend upon the permeability of the tissue to oxygen, the internal concentration of oxygen being the important factor. Although experiments have not been carried out with *Helianthus,* the results obtained with similar tissues of other plants indicate that an external concentration of 21 per cent. is sub-maximal, but variations in the concentration in this region only slightly affect the rate of carbon dioxide production. We have assumed that in using a constant external concentration of oxygen we have been getting a close approximation to a constant internal concentration, and therefore in view of what has been said above, that the results will be comparable.

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* Having already decided to express growth per unit of dry-weight, it is necessary to use the same unit when dealing with respiration, in order to make the results comparable.

With regard to the concentration of respirable material, if the rate of respiration remains constant, other factors being constant, it is probably safe to assume that the concentration of respirable material is in excess. Respiration expressed per gramme dry-weight per hour, when measured with the respirable material in excess, with the external concentration of oxygen that of the atmosphere, and with the temperature at 10° C., we propose to call the respiratory index, and from what we have said above, the respiratory index may be taken as a measure of the effective amount of respiring cell-matter per gramme dry-weight, or in other words, a measure of the "internal" factor.†

3. *Material and Methods.*

A detailed account of the plants used and of the manner in which they were grown will appear in the further papers of this series. In the present communication it will suffice to state that the variety of *Helianthus annuus* used was Sutton's "Giant Yellow" and that the plants were grown in the field under natural conditions of illumination and temperature and under optimal conditions of spacing and soil humidity. The seeds were sown on May 22, 1920, and the seedlings appeared above the surface on the 27th. While the plants were small the output of carbon dioxide was determined by absorption with baryta in Pettenkofer tubes, but later in the case of the larger plants the carbon dioxide was absorbed by NaOH in Reiset towers. The plants were cut off at the level of the ground. The plants used for respiration determinations were gathered towards the end of the day so that they should be taken after a period of active assimilation. Sufficient time was allowed for the tissue to cool down to the temperature of the respiration chamber. Whilst the plants were cooling down air deprived of carbon dioxide were drawn over at the same rate as that at which it was to be carried over during the experiment. The vessels used were always as small as the size of the material would permit in order that the time interval between the evolution of carbon dioxide from the tissues and its absorption by the baryta or soda should be minimal and that the current necessary to keep the carbon dioxide-content of the air in the vessel low should not be excessive. To this end it was found very suitable to enclose the plant material in a flexible air-proof fabric, since a covering of such a fabric collapses when air is drawn through it and clings tightly to the plant material. Until the plants were about 10 grm. in weight they were used entire, but subsequently the respiration of the stem, leaves and flowers was measured separately.

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* In the absence of any deeper knowledge of the process of respiration, the rate of carbon dioxide production is employed as a measure of the rate of the process.

† Palladin (7) has shown for seedlings that the respiration under constant external conditions, when carbohydrate is in excess, is proportional to the fraction of protein which is unattacked by gastric juice, thus suggesting that the amount of nuclein nitrogen is a measure of the amount of respiring cell-matter. This suggests that the respiratory index of the plant may be related to its nuclein nitrogen-content.

4. *Experiments to Determine the Relation between Temperature and Respiration, and a Suitable Temperature at which to Determine the Respiratory Index.*

It is known that at high temperatures the rate of respiration of plant tissues decreases after a short interval. This falling off in the rate of respiration has been attributed to a decrease in the effective amount of respiring cell-matter due to the high temperature ("time factor" of Blackman). The result may, nevertheless, in some cases be due to a decrease in concentration of respirable material with time. At medium and low temperatures it has been shown by Kuyper (5) for seedlings, and in this laboratory for leaves, that the respiration remains constant for an appreciable interval of time after separating a tissue from its source of carbohydrate supply.

With these considerations in view, a series of preliminary experiments was conducted to investigate the respiration of cut plants of *Helianthus* at various temperatures in order to ascertain for what length of time, after gathering, the respiration remained constant. The results provide at the same time data for the construction of a temperature-respiration curve.

Experiments were carried out with plants while young, *i.e.*, before the fourth pair of leaves had begun to develop. During this period, as will be shown later, the respiratory index changes little with age. Constant temperature rooms at low, medium, and high temperatures were utilised. The results are presented in Tables I to III and figs. 1 and 2.

Table I.—Respiration of *Helianthus annuus* at Low Temperatures.

No. of experiment.	Days from germination.	Interval between gathering and placing in temperature chamber.	Time in temperature chamber before estimations started.	No. of plants used.	Dry-weight of an average plant.	Temperature during experiment.	Duration of experiment.	Respiration (Mgrm. CO_2 per gramme dry-weight per hour).
		hours.	hours.		mgrm.	° C.	hours.	
1	12	2·5	6	20	197	3·5	41·2	1·28
2	14	0·75	2	12	184	1·8	15·2	1·042
3	15	0·5	4·75	10	180	1·0	14·6	0·97

With regard to the respiration at 25° C., it will be seen that the rate falls continuously after the first period of measurement, and that, as far as our results show, the initial rate of respiration can only be determined approximately at this temperature by extrapolating the curve. In estimating the initial rate of respiration at 25° C., we have assumed that the falling off has been continuous. At 10° C. no falling off occurred for about twelve hours. After this period the rate of respiration falls off, as is shown in Table II, Experiment 3. Presumably, in the case of older plants, where the amount of respirable material relative to respiring cell-matter is greater, the period before the falling off commences would be longer, and such was actually observed. Having found that at 10° C. there is no falling off for several hours in the rate of respiration, we have used that temperature throughout and have adopted it as the standard temperature of the respiratory index. It will be seen later that the index may range from 3·0 to 0·08 for different parts and ages of the plant.

At this point, the evidence which justifies the application of the temperature-respiration relation to the calculation of the respiration under the conditions of fluctuating temperature obtaining in the field may be considered.

At an early stage in the life of the plant, the actual respiration at the fluctuating temperature obtaining in the field was determined continuously for eight days (June 10, 1920, to June 18, 1920), fresh plants being used every half day.

The results of these determinations of the actual respiration were

Table II.—Respiration of *Helianthus annuus* at Medium Temperatures.

No. of experiment.	Days from germination.	Interval between gathering and placing in temperature chamber.	Time in temperature chamber before estimations started.	No. of plants used.	Dry-weight of an average plant.	Respiration (Mgrm. CO_2 per gramme dry-weight per hour).
		hours.	hours.		mgrm.	
1	1	0·25	2	30	22·5	3 hrs. at 10° C. 3 hrs. at 10° C. 2·9 → 2·8
2	2	0·75	3·5	25	22·3	13·5 hrs. at 10·4° C. 3·1
3	4	0·25	3	25	24·2	3 hrs. at 10° C. 17 hrs. at 10° C. 3·0 → 2·1
4	12	0·5	4·3	10	101	4 hrs. at 10·2°. 3½ hrs. at 10·1°. 6 hrs. at 9·9°. 2·7 → 2·8 → 2·5
5	21	0·5	3·25	8	630	2 hrs. at 11·1° 2 hrs. at 11·2°. 2 hrs. at 11·3°. 2 hrs. at 11·4°. 3·3 → 3·2 → 3·4 → 3·3

Table III.—Respiration of *Helianthus annuus* at High Temperatures.

No. of experiment.	Days from germination.	Interval between gathering and placing in temperature chamber.	Time in temperature chamber before estimations started.	No. of plants used.	Dry-weight of an average plant.	Temperature during experiment.	Respiration (Mgrm. CO_2 per gramme dry-weight per hour).
		hours.	hours.		mgrm.	° C.	
1	11	2·5	3	40	175	25	2½ hrs. 6·9
2	14	0·75	5·8	10	163	25	3 hrs. 3 hrs. 6·5 → 5·5
3	15	0·5	8·25	10	157	25	4 hrs. 4 hrs. 4 hrs. 5·5 → 4·6 → 4·0
4	18	0·5	0·8	4	485	25	3 hrs. 3 hrs. 3 hrs. 3 hrs. 8·2 → 6·9 → 6·3 → 5·8

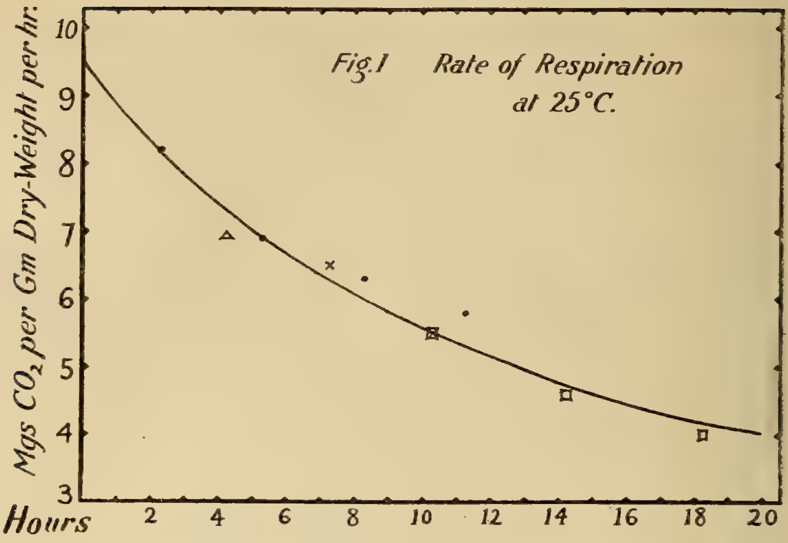

Fig. I Rate of Respiration at 25°C.

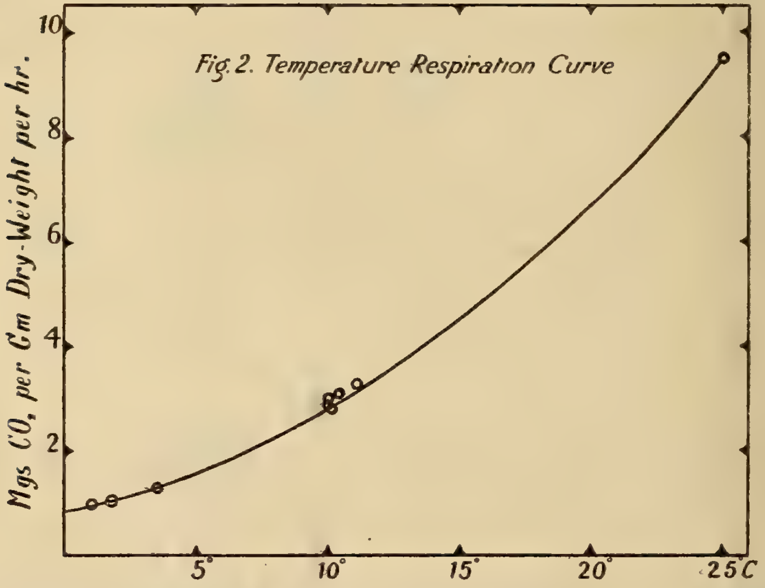

Fig. 2. Temperature Respiration Curve

compared with values calculated from the respiratory index determined at 10° C. on one occasion during the eight days. For the purpose of this calculation, the physiological mean temperature for each period was found from the temperature record.* Our method of obtaining this is as follows.

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* There are several possible methods of obtaining the average temperature for a period. The geometric mean temperature would be used in preference to the arithmetic mean temperature, because the rate of a physiological process is not a linear function of temperature. But since the temperature coefficient decreases with increase of temperature it is not sufficiently correct to use a geometric mean.

The recorded temperatures—in our case taken at hourly intervals—are tabulated. For each temperature, the rate of the reaction (*i.e.*, respiration) is obtained from the temperature-respiration curve. The arithmetic mean of these is taken, and the corresponding temperature obtained from the temperature-respiration curve.

The total observed production of carbon dioxide was 299 mgrm., whilst the calculated value was 326 mgrm. This agreement is good, considering the fact that a falling off in the rate of respiration must have occurred during the periods when high temperatures obtained. The results of the experiment, as a whole, show that a satisfactory allowance for fluctuating temperature can be made by means of the temperature-respiration curve, at least during the earlier stages of the life-cycle, to which the above experiment applies.

5. *The Decrease with Age in the Respiratory Index of the Plant and of its Parts.*

We may now proceed to the main issue, namely, the determination of the respiratory index of the whole plant and of its parts throughout the life-cycle.

The results of the actual experiments are given in Table IV.

Table IV.—Observed Respiratory Index of Plants of Different Age.

Days from germination.	Number of plants used.	Dry-weight of a single plant.	Respiratory index (mgrm. CO_2 per gramme dry-weight per hour) of				Total inflorescences.	Flowers on lateral shoots.
			Entire plant.	Stem.	Leaves.	Stem-apex.		
		grm.						
1	30	0·0225	2·90					
2	25	0·0223	3·00					
4	25	0·0242	3·00					
13	10	0·1009	2·80					
22	8	0·630	3·00	(3·00)	(3·00)	3·00		
29	2	4·065	2·30					
36	1	12·85	1·21	0·81	1·56			
43	1	22·05	1·03	0·69	1·38			
50	1	45·15	0·94	0·46	1·52	2·56		
59	1	93·20	0·66	0·33	1·32	1·72		
64	1	98·30	0·71	0·34	1·24			
89	1	294·7	0·48	6·31	0·90	1·13*	1·13	
99	1	377·4	0·37	0·25	0·45	0·89	1·04	1·31
112	1	818·3	0·26	0·098	0·375	0·75	0·85	0·95
136	1	419·5	0·39	0·081	0·44	0·96	0·965	0·97

* From this date onwards the stem-apex was the inflorescence only.

In the first place, with regard to the plant as a whole, in applying these results for the purpose for which they were intended, namely, for determining variations with age in the value of the respiratory index and for calculating the respiration in the field of a mean plant, the objection may be raised that no allowance is made for the probable error of the respiration value determined from a single plant. It is true that it would have been better to have determined simultaneously the respiration of several individual plants, and from the results obtained to have calculated the mean result with its probable error. Such a procedure was impossible with the facilities at our disposal. We can, however, apply the following correction to our figures. Probably a principal cause of any extensive difference between the respiratory index of one plant and that of another is the difference in the relative proportion of stem to leaf, when the respiration of the stem differs from that of the leaves. Knowing the difference between the relative proportion of stem to leaf in the plant used for determining the respiration and that in a mean plant, and having after the thirty-fourth day from germination determined the respiration of the stem and leaves separately, we have from this date onwards been able to make an allowance for the effect of a difference in proportion of stem to leaf in calculating the respiratory index of a mean plant. This correction, however, makes very little difference. The figures for the respiratory index of a mean plant of the harvest are given in Table 5.

Table V.—Calculated Respiratory Index of an Average Harvested Plant.

Days from germination.	Dry-weight of plant.	Respiration per hour at 10° C. of an average plant.	Respiratory index of an average plant (mgrm. CO_2 per gramme dry-weight per hour).
	grm.	mgrm. CO_2.	
1	0·0238	0·071	2·9
8	0·0707	0·212	2·9
22	1·128	3·38	3·0
29	4·53	10·4	2·3
36	12·35	14·9	1·2
43	26·9	27·7	1·03
50	60·1	56·1	0·93
57	92·8	68·8	0·74
64	133	91·5	0·69
85	352	176	0·53
99	559	198	0·35
113	765	233	0·31
127	873	345	0·39

It will be seen from the above Table that the value for the respiratory index of the mean plant falls off continuously from 3 to about one-tenth of that amount at the end of the life-cycle.

When in the second place we consider the respiration of the various parts of a plant, such as the stem, leaves, and flowers, we find a similar phenomenon of a decrease in the respiratory index with age. Table IV shows a fall in the value of the respiratory index of the stem from 0·8 on the thirty-sixth day from germination to 0·08 on the 136th day from germination, and, in the value of all the leaves taken together, from 1·56 to 0·44.

Table VI.—Respiration of Leaves of *Helianthus annuus.*

Days from germination.	Description of leaves used.	Number of leaves used.	Weight of leaves used.	Weight of an individual leaf.	Respiratory index (mgrm. CO_2 per grm. dry-weight per hour).
			grm.	grm.	
22	Seedling plant (minus roots)	—	0·630	—	3·00
50	Top cluster and one or two small leaves ...	—	4·65	—	2·56
	The remaining leaves of the plant	—	16·00	—	1·21
53	Top cluster of leaves: A + flower rudiments	—	4·76	—	1·84
	B — flower rudiments	—	2·64	—	1·80
54	2nd pair from bottom of plant (excluding cotyledons)	12	5·85	0·49	0·89
	3rd pair do.	12	11·35	0·95	0·89
	4th pair do.	12	17·40	1·45	1·00
	5th pair do.	12	21·90	1·83	1·02
	Two pairs next to top cluster	24	23·50	0·98	1·81
	Top cluster (flower rudiments just appearing)	—	11·10	—	1·83
59	Top cluster of leaves (including flower rudiments)	—	3·40	—	1·72
	Remaining leaves of plant	—	27·80	—	1·27
64	Upper (*i.e.* younger) leaves of the plant ...	—	1·96	—	1·70
	Remaining leaves of plant	—	31·70	—	1·22
89	All the leaves of the plant	—	79·00	—	0·905
99	Leaves off the lateral branches	—	14·70	—	1·11
	Leaves off main stem	—	72·50	—	0·32
112	Leaves off the lateral branches	—	44·50	—	0·60
	Upper leaves off main stem	—	77·90	—	0·365
	Lower leaves off main stem	—	90·50	—	0·27
136	All the leaves of the plant	—	78·50	—	0·44

The decrease in the rate of respiration of all the leaves taken together is undoubtedly an expression of the fact that the respiratory index of individual leaves falls off with age—a fact previously established by Nicolas (6).*

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* Nicolas (6) showed that, broadly speaking, the rate of respiration of leaves is dependent upon their age; but this investigator did not distinguish between the changes in the rate of respiration due to differences in the age of the leaf and those due to differences of the age of the plant.

Inspection of Table VI shows that, on the twenty-second day from germination, the value of the respiratory index of the young plant with three or four pairs of leaves was about 3; on the fifty-fourth day from germination, the values for the second, third, and fourth pairs of leaves (from the bottom of the plant) were 0·89, 0·89, and 1·0 respectively. On the 112th day from germination, on which date the three lowermost pairs of leaves had fallen, the value of the respiratory index of those remaining on the lower half of the stem was only 0·27.

A similar decrease has been observed in the case of the flowers, whether the main inflorescence alone or whether all the flowers of the plant be considered.

We come finally to the results obtained with the actively growing stem-apex. The case here has the peculiar interest that we are dealing with a tissue which, unlike the plant as a whole and the parts previously mentioned, is not complicated by increasing differentiation with age, but is still mainly meristematic and might therefore be expected to retain its original respiratory activity. The respiratory index at the stem-apex is not, as might be expected, constant throughout the life of the plant, but falls continuously as the age of the plant increases. The stem-apex may be regarded as giving us the initial respiratory index of each leaf as it appears; that is, approximately the respiratory index of the meristematic tissue.

In Table VI it is seen that on the twenty-second day from germination the value of the respiratory index of the top cluster of leaves (in this case the entire seedling plant) was 3. On the fiftieth day from germination the value for the respiratory index of the top cluster of leaves was 2·56, whilst on the fifty-ninth day from germination it had fallen to 1·72. After this date the inflorescence appeared, but, as will be seen from the same Table, on the fifty-third day from germination the value for the respiratory index of a top cluster of leaves without visible flower rudiments was the same (1·80) as that for a top cluster of leaves from another plant in which the young inflorescence had developed.

In concluding this section we may consider the decrease with age in the respiratory index of the whole plant with a view to separating the component factors which we have until now grouped together as the "internal" factor. It will be seen from Table IV that during the first fifty-nine days from germination, after which period the inflorescence appeared, the greatest fall in the value of the respiratory index occurs in the case of the stem, whereas the fall is smallest in the case of the stem-apex. If we assume that the value for the respiratory index of the stem, leaves, and stem-apex is the same just after germination—the fact that the respiratory index of the entire plant does not change appreciably for a period extending over more than three weeks from germination being good evidence that this is the case—we find that the fall in the respiratory index during this period of the stem, leaves, and stem-apex is 89, 56, and 43 per cent. of the original value respectively. The fall in the case of the stem and leaves must be partly due to the increasing proportion of definitely non-living tissue, such as mechanical and water-conducting tissues, but this cannot be the explanation of the fall in the respiratory index of the stem-apex. The fact that the respiratory index of the youngest leaves falls with the age of the plant is interesting and calls for an explanation in itself. On our postulates the effective amount of respiring cell-matter per gramme dry-weight in the young leaves becomes smaller as the age of the plant increases. As to whether this is due to a decreasing amount of protoplasm or respiring enzymes per gramme dry-weight, or to an increasing amount of some inhibitor, remains an interesting question, to be answered by further research. It is interesting to note, however, that the photosynthetic activity of the young leaves also falls off with increasing age of the plant.* A knowledge of the protoplasmic nitrogen-content of the whole plant and of its parts, particularly that of the young leaves, would possibly throw light on the causes underlying the fall with age in the respiratory index.

6. *Respiration oj Uncut Plants in the Field.*

The following experiments wth uncut plants in the field are of interest in that they confirm the results already described, which were obtained with cut plants in the laboratory. On five occasions, scattered throughout the life-cycle, the respiration of an uncut plant was determined *in situ* in the field. The leaves of the plants were drawn up to the stem and secured with string; an airtight fabric covering was then placed over the plant. This covering was made airtight at the base by means of a waxed joint holding water. The top of the covering was tied and also sealed with wax. The inlet was a long narrow tube at the top, the outlet being at the bottom. A current of air was drawn over the plant and thence through a gas meter. The carbon dioxide was absorbed by caustic soda in a couple of Reiset towers. Allowance was made for the carbon dioxide present in the air at the rate of 3 c.c. per 10 litres. The physiological mean temperature was taken and the respiration results were reduced to 10° C. by means of the temperature-respiration relation previously established. The results are presented in Table VII, in which are also recorded the respiratory indices for the dates in question.

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* Unpublished work by G. E. Briggs.

Table VII.

Dates and times of experiment.	Dry-weight of a single plant.	Physiological mean temperature.	Mgrm. of CO_2 per gramme dry-weight per hour calculated for 10° C.	Respiratory index for mean plant.
	grm.	° C.		
5 P.M., June 26, to 10.5 A.M., June 27 ……	5·68	16·0	1·58	2·00
4.20 P.M., July 15, to 7.10 A.M., July 16 ……	51·4	12·6	1·28	0·96
4.3 P.M., July 19, to 12.10 P.M., July 20 ……	62·5	15·3	0·94	0·97
2.3 P.M., Aug. 17, to 8.26 P.M., Aug. 17 ……	227	16·7	0·69	0·58
7.32 P.M., Sept. 11, to 9.34 A.M., Sept. 12 …	354	6·8	0·26	0·30

The evidence from uncut plants in the field corroborates that derived from cut plants in the laboratory to the extent that there is continuous falling off in the respiration with age, and, moreover, the values given are of the same order. The agreement is not very close, three being higher and two lower than the respiratory index of the average plant at the same date. In view of the following considerations, a close agreement is not to be expected. Whilst in the case of the laboratory experiments the temperature of the tissues was presumably that of the chamber, since suitable precautions were taken, in the case of the field experiments there must have been a considerable lag before the cooling down or warming up of the bulky tissues was accomplished. Again, whilst in the laboratory the volume of the respiration chamber was minimal, the airtight fabric collapsing closely round the plant tissue, such a condition was impossible in the field without unduly damaging the plants. Further, by tying the leaves up they were subjected to a continuous geotropic stimulus, which was exhibited when the plants were untied by a decided downward movement of the leaves, which took up a fixed position with the petioles pointing almost vertically downwards. Another possible source of error attaching to the field experiments lies in a possible inaccuracy in the allowance made for the carbon dioxide-content of the air; but this, however, could only slightly affect the results. Finally, the proportion of leaf to stem was not that of a mean plant and could not be allowed for, since the respiration of the leaves and stem could not be determined separately.

7. *The Relation of the Respiratory Index to Relative Growth Rate.*

The relative growth rate, which we have elsewhere (8)* termed R., is the average rate of increase in dry-weight per unit dry-weight per week (expressed as a percentage). In previous papers (1 and 4) we have shown that the relative growth rate appears to vary with age in a manner slightly different for different plants, but to be always characterised by a fall beginning at an early period in the life cycle; this fall is very similar to the fall which we have recorded above in the values of the respiratory index of *Helianthus annuus.* In the present research we can make a direct comparison between the respiratory index, which is the rate of respiration per unit dry-weight, and R., since we have the results for the relative growth rates of the same crop of plants as that used for determining the respiration. The comparison is represented in fig. 3 and Table VIII. Fig. 3 shows three curves:—The relative growth rate values, the values for the respiratory index and the values for the respiratory activity at the average temperature obtaining in the field, that is, the respiratory index corrected to the average temperature in the field by means of the temperature-respiration relationship established above, plotted in each case against days from germination. Table VIII also gives the ratios of relative growth rate to the respiratory index and to the respiratory activity at the average temperature obtaining in the field.

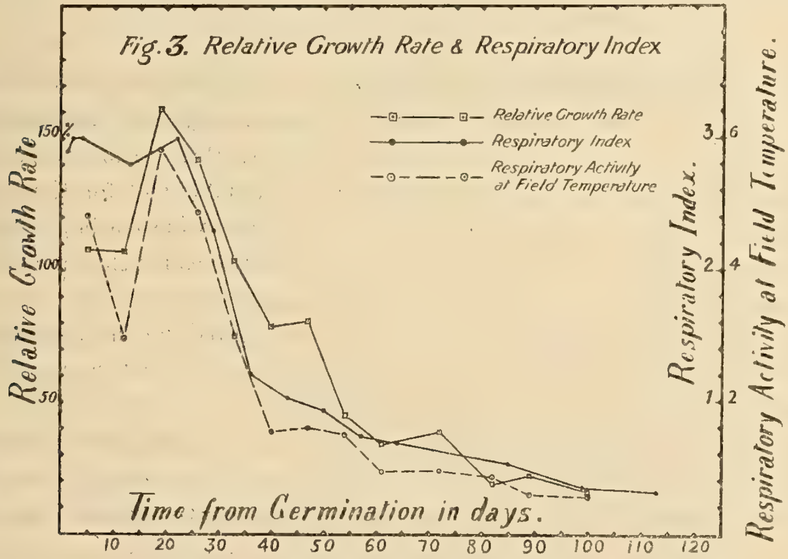
Fig. 3. Relative Growth Rate & Respiratory Index

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* $\frac{R}{100} = \log_e W_2 - \log_e W_1$, where W_2 and W_1 are the dry-weights at the end and beginning of the week respectively, and e the base of the natural logarithms.

Table VIII.—Relation between Growth Rate and Respiratory Activity.

Days from germination.	Relative growth rates.	Respiratory index.	Ratio of relative growth rate to respiratory index.	Respiratory activity at average field temperature.	Ratio of relative growth rate to respiratory activity at average field temperature.	Physiological mean temperature in the field.
5	108	2·97	0·365	4·85	0·223	15·1
12	107	2·82	0·38	2·97	0·360	10·5
19	161	2·92	0·55	5·84	0·275	17·6
26	142	2·60	0·55	4·87	0·291	16·8
33	104	1·65	0·63	3·09	0·336	16·6
40	79	1·10	0·72	1·58	0·500	13·6
47	81	0·97	0·84	1·63	0·496	15·4
54	45	0·82	0·55	1·52	0·296	16·6
61	34	0·72	0·47	0·94	0·361	12·8
72	39	0·63	0·62	0·97	0·402	14·4
82	19	0·53	0·36	0·90	0·211	15·1
89	22	0·47	0·47	0·62	0·355	12·5
100	16	0·38	0·42	0·52	0·308	14·0
114	4·5	0·32	0·14			

It will be seen that while the relative growth rates vary over a wide range, the ratio of the relative growth rate to the respiratory index remains fairly constant throughout the life-cycle. In considering these ratios it should be borne in mind that the values for the respiratory index and for the relative growth rate are not strictly comparable for the following reason. Whereas the values for the respiratory activity are maximal values for the temperature, whether 10° C. or the average temperature of the field, the values for the relative growth rate may not be, but may be limited by the operation of uncontrolled factors, such for example as supply of carbohydrate or of inorganic salts.

In order to be strictly comparable, whether *inter se* or with the respiratory activity, the values of the relative growth rate should be maximal, that is, not limited. Further, the respiratory activity and the relative growth rate, both being maximal at field temperatures, would be comparable throughout the life cycle only if the effect of temperature upon the relative growth rate and upon the respiratory activity were the same. In other words, for purposes of strict comparison the respiratory activity and the relative growth rate should not only both be maximal values but should both be determined at the same constant temperature throughout the life-cycle. Since we do not know the relation between temperature and growth the legitimacy of correcting the respiratory index values determined at 10° C. up to field temperatures for comparison with the relative growth rate is doubtful. As a result of this correction, however, the ratio of relative growth rate to respiratory activity at field temperatures is seen to vary with one exception inversely with temperature. In view of this and of the fact that the correlation coefficient of this ratio with temperature is −0·34, it would seem either that the relative growth rate (when not limited by such factors as carbohydrate or salt supply) has a lower temperature coefficient than respiration, or that the relative growth rates at higher temperatures are limited by some factor.

In view of the close relation between the respiratory index and nuclein nitrogen, as established by Palladin (7), on the one hand, and that between the respiratory index and the relative growth rate, as demonstrated above, on the other, one would expect the growth rate to be intimately connected with the concentration of nuclein nitrogen per gramme dry-weight. It must be realised, however, that whereas the first relation was established for wheat, the second was established for *Helianthus annuus.*

The only type of evidence available with regard to the third suggested relation is that provided by the results obtained by Hornberger (3), who determined the protein nitrogen-content of maize. These results are for the same plants as those for which we have already calculated the relative growth rate (1). The growth per protein nitrogen for successive weeks, which we have calculated, is as follows:—

Grammes dry-weight per gramme Nitrogen per Week.

22·0	32·4
42·5	20·0
25·9	15·2
27·7	16·2
38·1	27·5

As to the relation of nuclein nitrogen to protein nitrogen we at present have no evidence.

It is proposed to reserve further consideration of the relation between respiration and growth until we have presented the growth data in full.

8. *Summary.*

The respiratory index has been defined as the respiration determined per gramme dry-weight per hour at 10° C. when the amount of respirable material is not limiting and when the external concentration of oxygen is that of the atmosphere. The respiratory index is consequently a measure of the effective amount of respiring cell-matter per gramme dry-weight, that is the "internal" factor for respiration.

The respiratory index of the entire plant falls off continuously from 3 to about one-tenth of this value at the end of the life-cycle. The respiratory index of the stem of individual leaves and of the flowers respectively decreases with the age of the organ. The initial respiratory index of successive leaves—*i.e.*, the respiratory index of the stem-apex—decreases with the age of the plant, indicating that the respiratory index of the meristematic tissue decreases with age. The fall in the respiratory index of the meristematic tissue and young leaves shows that the fall in the respiratory index of the whole plant is not, as might be expected, due entirely to the increase in proportion of such tissues as mechanical and water-conducting tissue.

The fall in the value of the respiratory index with age follows closely that in the value of the relative growth rate, thus indicating a close connection between the "internal" factor for respiration and the "internal" factor for growth.

In conclusion, the authors wish to express their thanks to Mr. P. Parija and Mr. E. J. Maskell for carrying out titrations, and to Dr. F. F. Blackman, F.R.S., for ever ready advice and stimulating criticism.

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Idio-Ventricular Periodicity.

By D. H. de Souza and J. A. Hewitt.

(Communicated by Prof. W. D. Halliburton, F.R.S. Received September 30, 1921.)

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When the excised heart of the frog is perfused under certain conditions, the beats lose their normal regularity, and occur in periodic groups with a long pause between every two groups. This phenomenon was first investigated systematically by Luciani* in 1872, and, in consequence, the groups are often referred to as "Luciani groups." They are not infrequently seen in experiments in which a Symes cannula, inserted into the heart through the inferior vena cava or sinus venosus, is kept in place by a ligature tied between the sinus venosus and the auricles, in the position of the first Stannius ligature. When the ligature is tied, the auricles and ventricles cease to beat, but if the heart is excised and then perfused through the cannula by Symes's method,† the whole heart usually beats again, and the beats may show periodic grouping before settling down to a normal regularity. Sometimes, too, groups occur in hearts in which the perfusion by Symes's method has been going on for some time and the heart has been beating regularly.

The conditions of occurrence of the groups have been studied by several physiologists, and the general opinion seems to be, like that of Gaskell,‡ that they are due to an asphyxial state of the heart, that the pauses indicate a complete heart block, and that the groups of beats occur when the block has become only partial, so that some beats are able to get through from sinus venosus to auricles and ventricles.

In the course of some perfusion experiments which we have been carrying out, we obtained a tracing which, it seems to us, cannot be explained in this way. As it illustrates a phenomenon in connection with periodic grouping of which we can find no description, we put it on record in this note.

Experiment.—A cannula was inserted into the heart of a pithed female frog through the inferior vena cava, and the heart was secured to the cannula by a ligature passing round it in the groove between the sinus venosus and the auricles, and not including the aortic trunk. The heart, attached to the cannula, was excised and perfused by Symes's method, with Ringer's fluid (NaCl 0·6 per cent., KCl 0·026 per cent., $CaCl_2+6H_2O$ 0·032 per cent.), at a pressure of about 4 cm. of water, and a tracing of the beats was taken by means of a lever, connected by a hook to the tip of the ventricle, and writing on the smoked surface of a revolving drum. Shortly after the perfusion was begun, the heart beats were normal and regular. As soon as it was evident that this normal regularity was established, the perfusion fluid was replaced by Ringer's fluid, containing *i-inositol* (1 in 750). In less than a minute after the inositol solution reached the heart, the beats showed periodic grouping. The heart was perfused with the inositol solution for 13 minutes, then with ordinary Ringer's solution for half-an-hour, and during the whole time grouping continued. After this the perfusion was ended by the solution's running out. Grouping ceased, and the heart beat regularly, the beats gradually diminishing in force and frequency. At this stage the experiment was concluded.

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* Luciani, 'Human Physiology' (translated by F. A. Welby), vol. 1, p. 302 (1911).

† Symes, 'Proc. Physiol. Soc., Jour. Physiol.,' vol. 43, p. xxv (1912).

‡ Gaskell, 'Text-book of Physiology' (edited by E. A. Schafer), vol. 2, p. 226 (1900).

Interpretation of the Tracing.—Fig. 1, taken from a part of the tracing, is a type of the whole. It shows the ventricle beats arranged in groups, with pauses between the groups. The auricle beats are clearly seen and continue to occur at regular intervals during the ventricular pauses. Dr. Locke, who happened to be present while the experiment was in progress, drew our attention to the fact that, in the groups, the ventricle beats did not seem to bear a definite time relationship to the auricle beats. This is evident in the tracing, where, during the grouping, the contraction of the ventricle occurs at a variable time after that of the auricle, and may even synchronise with it. We have carefully measured and analysed this tracing, and we submit in fig. 2 a diagram which illustrates the essential points.

The vertical lines in the upper half of the diagram indicate the relative positions of the contractions of the auricles, those in the lower half the relative positions of the contractions of the ventricle during the same period of time. The intervals between the auricle contractions are practically equal; accordingly, when the auricle beats are obscured on the tracing by ventricle beats, we have inserted broken lines at this interval from the lines preceding and following them. Two ventricular pauses are shown, and a complete group between them.

It is evident that there is no definite time relationship between the contractions of the auricles and of the ventricle in this group, such as can be explained by a condition of partial heart block, and this is the same for all the groups; in fact, it is obvious that, during a part of the time, the ventricle was beating more frequently than the auricles.

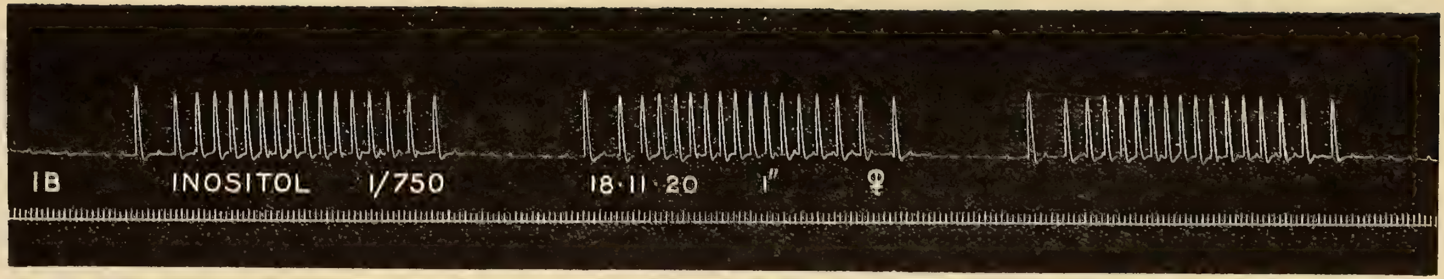

FIG. 1.—Tracing showing periodic grouping of the beats of the ventricle of the frog's heart. For explanation see text.

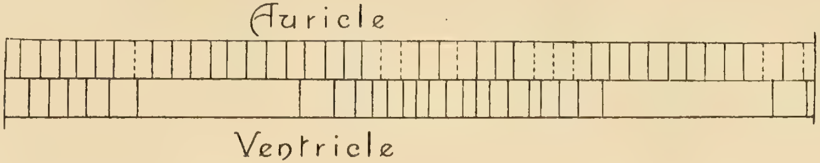

FIG 2.—Diagram showing grouping of the ventricle beats, and the order in which the auricle beats and ventricle beats occur. For explanation see text.

The simplest explanation that occurs to us is that there was a complete heart block between the auricles and ventricle, the former continuing to beat regularly; and that the periodic grouping does not indicate a partial removal of the block but was a ventricular phenomenon; in other words, that this is an instance of idio-ventricular periodicity.

It must be understood that we do not advance this explanation for all cases of periodic grouping; all other tracings that we have seen are able to bear the usual interpretation. Moreover, it is beyond the scope of this note to discuss to what extent, if any, the result may have been due to the action of inositol.

Summary.

A perfusion experiment on the excised heart of the frog is described and discussed, in which periodic grouping occurred as an independent ventricular phenomenon.

OBITUARY NOTICES

OF

FELLOWS DECEASED.

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PAUL EHRLICH, 1854—1915.

It is fitting that some account should be given, in the publications of this Society, of a Fellow so eminent in Science and of an influence so great as Paul Ehrlich. We shall first give the chief facts of his life and afterwards consider the nature and significance of his work.

Ehrlich was born in 1854 at Strehlen, a small town in Silesia. He was of Jewish extraction, like so many others who have risen to fame, and was a cousin on his mother's side of Carl Weigert, with whom he enjoyed an intimate and valuable friendship, lasting till Weigert's death. Ehrlich's early education was received in his native place, and afterwards in the Gymnasium of Breslau. At Breslau also he attended the University for a semester and then went to Strasburg, where he took up the study of medicine. Amongst his teachers there were Waldeyer, the anatomist, whose attention he attracted by his application of aniline dyes to the staining of tissues, and Biermer, the distinguished physician. After completing his curriculum, he worked for a year in the Pathological Institute, under the stimulating direction of Cohnheim and Heidenhain, and in association with Weigert, Salomonsen, and Welsh. There is little evidence, however, that he was much swayed by the influence of any one man; certainly his field of work was chosen and laid out by himself.

In 1878 he went to Berlin, to take up his duties as chief assistant in Frerich's clinique. There he worked for several years and published his papers on hæmatology (which were the first to establish his reputation) and on *intra-vitam* staining, etc. The growing science of bacteriology naturally presented many problems which appealed to his bent of mind; but when engaged with these, he found, in 1888, that he had contracted tubercle and he had to give up work for a time. Fortunately, a satisfactory cure was effected and he was enabled to resume his researches, which were now to be occupied for some years with questions of toxic action, immunity, etc.

Returning to Berlin in 1890, he worked in a laboratory of his own for a time, but afterwards obtained a post in the newly established Institut für Infectionskrankheiten. In 1896, on the establishment of the new Serum Institute, he was appointed Director on the recommendation of Althoff, the Prussian State Minister, who early recognised his genius. When the Institut für Experimentelle Therapie was established at Frankfurt, in 1899, he was transferred to its directorship. His earlier work had been done almost single-handed, but we now see him surrounded by a large body of able workers, and equipped for his requirements in a manner which at that time had hardly been equalled. He continued his researches on immunity and instituted on a large scale experimental investigations on cancer. The latter were not of his own choosing, but were taken up at the request of the Government. He was, however, fortunate in being able to carry on contemporaneously that remarkable series of researches in chemo-therapy which culminated in his discovery of salvarsan in 1910. He died suddenly in August, 1915, in the full height of his mental activity and vigour.

Ehrlich's earlier work, belonging as it did to no distinct branch of science, did not for a time receive the attention it merited, but the importance of his researches on immunity could not be ignored, though his views involved him in much controversy, sometimes not devoid of bitterness. It was, however, his great therapeutic discovery that set him on a pinnacle in his own country, and from this time he was acclaimed as one of the greatest. The recognition of his work in this and other countries was a great gratification to him. He was Croonian Lecturer in 1900, he gave the Harben Lectures in 1907, and he delivered a remarkable address on chemo-therapy at the International Medical Congress in London in 1913, meeting with an ever-growing welcome and appreciation. In 1908 he was awarded, jointly with Metchnikoff, the Nobel prize, and in connection with this he delivered an address on "The Partial Functions of the Cell," which contains the latest development of his views on the receptor-apparatus of cells.

Although Ehrlich thus worked in and enriched various fields of science, it is not difficult to trace a unifying principle throughout his investigations. One subject led to another; his work as a whole is an evolution. This leading principle is the affinity of the constituent molecules of living matter for various chemical substances brought into relationship with them. To treat the substance of cells according to the principles of the organic chemistry of the day did not lack in boldness, and if we waive the question as to the correctness of theory, we must admit that the results were striking. This principle in Ehrlich's work is seen first when he was a student of medicine, in connection with a paper on lead poisoning by Heubel, who claimed that the organs in which the lead accumulated had also after death the property of fixing the metal. Ehrlich recognised the difficulty of this problem of fixation and distribution by quantitative methods, and conceived the idea of using a coloured substance for the study of the problem, introducing fuchsin for the purpose with success. The period of his work at Berlin was largely occupied by this question of selective affinity, and though it supplied methods of great value to the histologist, one must recognise that a dye was employed simply as a suitable means in the study of the larger question. His duties in connection with clinical medicine, though not in themselves attractive to him, supplied problems of interest and also material for research, and in this way many of his investigations at the time came to be in connection with diseases of the blood.

By his method of "Farbenanalyse" he was for the first time able to recognise and differentiate classes of cells. He discovered the specific granulations of the leucocytes, classified these cells, and especially insisted on the fundamental difference between the lymphoid and myeloid tissues. It is still a matter of dispute in what sense this difference is to be accepted, but there is no doubt as to the fundamental importance of Ehrlich's observations both regarding the sources of leucocytes and their behaviour in reactive processes. Further, he published numerous papers on the anæmias, leucæmias, etc., and his observations still constitute the foundation of hæmatology. We owe to him also many important methods, *e.g.*, those for demonstrating glycogen in cells, for estimating the reaction of the blood, the diazo reaction, etc. And we owe to him also the demonstration of the acid-fast character of the tubercle bacillus and the method of demonstrating it, which is still in general use.

We have already indicated that Ehrlich's researches on stains had as their object not so much the obtaining of new histological methods, though these were supplied, as the throwing of light on the larger question of combining affinity, and it was accordingly natural that he should extend his principles to the living organism. The outcome of research in this domain was twofold, viz., the discovery of the *intra-vitam* method of staining, and his work on the oxygen requirements of the organism. By the injection of methylene blue into the circulation, or by placing a small living organism in the dye, he was able to demonstrate the processes of certain nerve-cells down to their finest endings. It is unnecessary to refer to later developments of this method, or to emphasise the importance which it has had in biological study.

His monograph, 'Das Sauerstoffbedürfnis des Organismus,' published in 1885, contains an account of experiments on the relative affinity of the tissues for oxygen. In these he employed two dyes, viz., alizarin blue and indophenol, both of which are reducible to leuco-compounds, the latter being the more readily reduced. On introducing one of these dyes, in a colloidal state, into the circulation of an animal, and killing the animal some time afterwards, he found that some organs were coloured blue, whilst others had reduced the dye and contained the leuco-product. Further, some organs which ordinarily did not reduce the dye did so when a state of asphyxia was established. By these methods he was able to deduce the relative reducing powers of different tissues for oxygen, and he explained the results on the supposition that there exist side-chains in the cell protoplasm, whose function is the fixation of oxygen for cellular needs, and that the affinity of these varies in different organs. In this work the germ of his side-chain theory is found.

The next period of Ehrlich's work was occupied chiefly by the study of the action of toxin and antitoxin. In 1891 he published papers on ricin and abrin, in which he showed that antitoxins to these vegetable toxalbumens could be produced by feeding certain animals with sublethal doses. Accordingly, antitoxin production was not peculiar to the case of bacterial toxins, nor was it essential that the poison should be introduced parenterally. He showed also that immunity to these poisons could be transmitted from the mother to the offspring, and that this was due to the direct passage of antitoxin from the blood of the former, chiefly through the milk; in other words, the immunity is of the passive order. From a study of toxins and their action, Ehrlich formed the view that a toxin has essentially a dual constitution, and that there are two essential factors in its action. It possesses an atom group, by which it is linked to the side-chains of the cell protoplasm, and a toxic group which produces the characteristic lesion or symptoms; and, by an extensive series of researches, he studied the changes occurring in a toxin in the process of deterioration. He early formed the view, and demonstrated by test-tube experiments, that antitoxin combines directly with and neutralises toxin—not by destroying its toxicity, but by satisfying its combining affinity, so that it no longer unites with the cell protoplasm; and it may be noted that the dissociation of toxin from antitoxin has since that time been demonstrated. He further supposed that toxin molecules resembled in constitution, or masqueraded as, foodstuffs, and were fixed to the cell protoplasm by its side-chains. Owing to their being lost for the purposes of the cell, they are cast off, still in combination with the toxin; the side-chains are reproduced, and, when the process is continued, they are reproduced in excess, and set free in the blood. These free side-chains or receptors are then in a position to act as antitoxin, combining with any toxin present, and preventing its union with the cells. Such, in brief, is Ehrlich's side-chain theory as evolved in the case of antitoxins; it was afterwards elaborated to explain more complicated anti-substances, agglutinins, lysins, etc.

We have seen how, in his earlier researches, Ehrlich demonstrated the selective affinity which definite chemical compounds have for the constituents of cells in the living or dead condition, whilst in antitoxic action there is an affinity which has within certain limits a specific character. This specific character he supposed to rest on the complicated structure or configuration of living matter, and the essential point in his side-chain theory is that anti-substances pre-exist in the cells, and become free as the result of the stimulus to over-production. The number of anti-substances is apparently without limit, and it may be objected that their pre-existence is inconceivable; but, in view of the established facts, the same criticism applies to any theory that can be put forward. The determination of the value of Ehrlich's side-chain theory will, however, be attained by future experimental work, not by discussion. The natural sequence to the work on antitoxin action was an extension of the enquiry to other anti-substances more complicated in their structure and mode of action, and the outcome at a later date was a long series of researches dealing especially with the lysins. These are of an intricate nature, but are essentially dominated by his previous views, and are characterised by imagination and ingenuity of plan.

Apart, however, from Ehrlich's theoretical contributions to this biological problem, his work on antitoxin led to practical results of the highest value. One of the chief features of his genius was the combination of imaginative outlook with the power of detailed working out; rarely has this combination been so remarkable as in his case. Standardisation of antitoxin was essential to successful therapeutics, and Ehrlich, in his capacity as Director of the Serum Institute, attacked the problem with his usual thoroughness.

He investigated in great detail the phenomenon of antitoxin production in the body, the nature of its neutralising action, and, what was a problem of great complexity, the changes which occur spontaneously in toxins. The last, in fact, were a bar to standardisation of toxin for practical purposes, but Ehrlich solved the problem by introducing a standard antitoxin, which, by means devised by him, could be kept practically unchanged. His method of standardisation has remained the one in general use down to the present day, and by means of it the method of antitoxin treatment has been stabilised and the value of the results enhanced.

Ehrlich's official work at Frankfurt, from 1901 onwards, included investigations into the nature of tumour growth, and these were carried out on a very extensive scale. Though no therapeutic results were reached, there followed many important additions to knowledge on the biological side, with regard to the conditions of growth and virulence of malignant tumours, the production of immunity, modifications of structure in relation to virulence, etc.; in fact, for a time, the work from Ehrlich's laboratory may be said to have largely dominated this field of research. The principles of immunity to bacterial disease were naturally brought to bear on the question, and methods were devised by which an analogous active immunity could be produced against a tumour otherwise invasive—the tumour, needless to say, being derived from another animal. The natural immunity possessed by an animal against a tumour from a different species is in a different category; it is due, not to destructive powers on the part of the animal, but to failure of the cancer cell to draw nourishment from the fluids of the host. The special feature of the cancer cell, according to Ehrlich, is an excessive avidity for nourishment, yet the cell fails to grow in any but the fluids of the species of animal from which it has come. Here again, just as in the case of anti-substances to proteins introduced parenterally, species-differences in molecular structure are brought out in a striking way.

To the failure of growth of the cancer cells in the conditions last mentioned, Ehrlich applied the term *athrepsy*, and described it as due to a want of correspondence between the cell receptors and the available food molecules. He analysed this athrepic immunity, and found that the same principles held in various bacterial and protozoal infections, as well as in chemotherapy, there being in all of them examples of the want of fixation as a preliminary to the necessary action.

It will be gathered from what has been said above, that Ehrlich drew a close parallel between the taking up of food molecules by a cell and the fixation to the cell of certain substances which act as poisons—both depend upon the presence of suitable side-chains or receptors in the cell protoplasm. Accordingly, the failure of a poison to act as such is often due to non-combination with the cell.

The application by Ehrlich of the principles just explained led to remarkable results. Using, in the first instance, trypanosome infections as the test, he found that a large number of substances had a marked parasiticidal action, one of the most striking effects being found in the case of trypan red, a single dose of which might cure a mouse otherwise fatally infected with the trypanosome of *mal de caderas.* In certain cases, after treatment with a drug, a relapse occurred, and he found that the parasites which had survived its action were no longer susceptible; in other words, they had acquired drug-fastness. That this had a chemical basis was shown by the fact that the fastness usually applied to substances of the same chemical group, but not to those of other groups, one of which included the arsenic compounds.

Amongst these last, atoxyl, which had been introduced as a trypanocidal substance by Breinl and Thomas, came to be studied, and what proved to be an important discovery was made, when Ehrlich, along with Bertheim, showed that this substance had not the constitution of an acetanilide, as was supposed, but was para-amino-phenylarsenic acid, a substance from which a great number of derivatives with different actions could be formed. For example, by introducing an acetic acid group, a substance, arsacetin, was obtained, which had a much greater effect on trypanosome infections, but was much less toxic to the tissues than atoxyl. Ehrlich thus came to formulate the view that the different atom groups, existing as side-chains in such substances, functioned in different ways; some were concerned in fixing the substance to the parasite or body-cell—were parasitotropic or organotropic —whilst others produced the toxic effect; but in every case combination or fixation was essential.

The problem thus came to be how to vary the structure of the chemical compound so as to produce maximum affinity for the parasite and minimum affinity for the cells of the body, along with the necessary toxic action on the former. Ehrlich further showed that the pentavalent arsenic, in which form it exists in the atoxyl series, has little direct parasiticidal effect until it is reduced to the trivalent form. He thus directed his attention to compounds containing trivalent arsenic, and found that their toxic action on the tissues was reduced when two molecules were combined by means of the arsenic group.

After the preparation and testing of a large number of substances, as indicated by the numbers, the goal of a satisfactory spirochæticidal substance was reached in dioxydiamidoarsenobenzol (No. 592), the hydrochloride of which is salvarsan (No. 606). In this substance the hydroxyl, aided by the amido groups, bring about the maximum affinity for the spirochætes, while, of course, the arsenic group leads to the parisiticidal effect. Although Ehrlich did not directly concern himself with practical medicine, no one appreciated more fully than he the precautions necessary for therapeutic success, and the importance of detail as regards administration. Was the drug really without harmful action on the tissues? Might not a drug-fast strain of spirochætes be developed? These and many other questions had to be answered, and a vast amount of experimental work was entailed. Ehrlich gave salvarsan to the medical word in 1910, and experience of the drug during the years since then has pronounced as to its value.

In a notice such as this it is impossible to do justice to many of Ehrlich's investigations;[*] his extraordinary industry is indicated by the several hundred papers which he published. We have selected only the main subjects, and have endeavoured to show their dependence on a common principle. Of his originality, of the extent and quality of his work, and of the practical results obtained, there can be, we think, only one opinion: they are all of the first order. From the outset he marked off a field of work for himself, and this was not confined to any one science, but encroached on the domain of several. A worker in biology, he called to his aid the services of chemistry, and his knowledge of both departments was immense. To what extent his application of purely chemical conceptions to certain vital processes was justified is a question which can be answered only in the future, but this does not affect the value of the actual attainments by his methods. Originality and boldness of conception are apparent in his earliest researches, and, as we have already indicated, his whole life's work is an evolution from these. His outlook was never utilitarian: "Science for its own sake" might have been his motto, and the practical fruits fell off incidentally, as it were. With remarkable imaginative power there was combined in equal degree the faculty of intensive work, and each problem was worked out by him down to minute details. The researches, guided by his master mind, which led up to the discovery of salvarsan, stand in a sense by themselves in the history of medical science. He saw that scientific investigations in certain departments must nowadays be "on a Dreadnought scale," as he himself put it in one of his characteristic phrases, and fortunately the great requirements of his later work were satisfied and success was attained. And apart from Ehrlich's actual discoveries, it must be recognised that at the beginning of the century there was no more potent and far-reaching influence than his in the domain of medical science.

R. M.

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[*] A full account and analysis of his work are given in the volume "Paul Ehrlich," a Festschrift published on the occasion of his 60th birthday in 1914.

S. SCHWENDENER, 1829-1919.

Simon Schwendener, who was elected a Foreign Member of the Royal Society in 1913, was a native of Switzerland. He was born on February 10, 1829, at Buchs, in the Canton of St. Gallen. His father was a farmer, but the son showed a preference for the pursuit of knowledge rather than for the practice of agriculture; consequently, on the completion of his school-education, he became, not a farmer, but a teacher in the elementary school of his native town. A bequest from his grandfather made it possible for him to enter upon a University career. With this end in view, he went to Geneva, where he studied Botany under Alphonse de Candolle. Unfortunately, his means were insufficient to enable him to complete his University course, and he was compelled to have recourse to school-teaching for a time. In 1856 he removed to Zurich, to resume his botanical studies under Oswald Heer, and on August 8 he took his degree with a phænological thesis, begun at Geneva, 'Ueber die periodischen Erscheinungen der Natur, insbesondere der Pflanzenwelt.'

Shortly before this Naegeli had come to Zurich, and, under his guidance, Schwendener began to study the microscopical anatomy of plants. So well did teacher and pupil agree that, when in 1857 Naegeli was called to the Chair of Botany in Munich, he took Schwendener with him as his assistant. After ten years with Naegeli at Munich, Schwendener was nominated Professor of Botany at Basle. Ten years later, in 1877, he moved on to Tübingen, where he succeeded Hofmeister; and in 1879, on the death of Alexander Braun, he became Professor of Botany at Berlin, where he remained for the rest of his long life. He died on May 27, 1919. He was never married.

The consideration of Schwendener's work may well begin with the important book 'Das Mikroskop,' in which he collaborated with Naegeli, published 1865-7 (2nd ed., 1877), a book which exercised considerable influence upon botanical thought. Naegeli wrote the part relating to the structure and morphology of plants, whilst Schwendener was responsible for the part dealing with the mechanism and the optical theory of the microscope. His remarkably efficient treatment of the subject revealed the natural bent of his mind towards mathematics, which was so marked that it is somewhat a matter of surprise that he did not take up physical science as his special study, rather than botany. At this time he was also engaged upon definitely botanical work, the only work of the kind that he produced, which made him famous. He had undertaken, no doubt at Naegeli's suggestion, an investigation into the structure of Lichens, the results of which, with the title "Untersuchungen ueber den Flechtenthallus," appeared in Naegeli's 'Beiträge zur wissenschaftlichen Botanik,' 1860-3-8. In the course of his work, he devoted special attention to the cells containing chlorophyll, which form an essential constituent of the thallus, the "gonidia," as they had been termed. It had long been known that the gonidia closely resemble certain free-living organisms which had been described as Algæ. Inasmuch as the gonidia were assumed to be developed from the colourless filaments of which the thallus mainly consists, the view was held that many of the simpler Algæ were in reality nothing but the gonidia of Lichens, which had become free and had continued so to live. With regard to the rest of the thallus, the similarity between the colourless filamentous tissue and the mycelium of Fungi had been recognised, as well as that between the spore-bearing fructifications of the Lichens and those of the Ascomycetous Fungi. But no definite idea as to the nature of the Lichen-thallus had been reached. Then it began to be realised that there might be another, inverse, interpretation of the nature of the gonidia which might lead to some satisfactory conclusion. The position was well stated by De Bary in the following passage taken from his 'Morphologie der Pilzen, Flechten, etc.,' 1866. Speaking of certain Lichens, he says: "The Lichens in question are either the fully developed fructifying states of plants, the incompletely developed forms of which have hitherto been regarded as belonging to the algal groups Nostocaceæ and Chroococcaceæ—or these Nostocaceæ and Chroococcaceæ are typical Algæ, which acquire the form of Lichens, because they are invaded by certain parasitic Ascomycetous Fungi, the filamentous mycelium of which penetrates into the developing thallus, and often becomes attached to the coloured cells." In the last of his papers published (1868) in Naegeli's 'Beiträge,' Schwendener, as the result of his observations on the gonidia, expressed himself strongly in favour of the latter of the above alternatives, and promised a further statement on the subject as soon as it became possible for him to resume his work, which had been interrupted by his removal to Basle. This promise he redeemed by the publication, in 1869, of his celebrated pamphlet "Die Algentypen der Flechtengonidien," in which he adduced convincing evidence that the gonidia do not originate in the thallus as developments of the filamentous tissue; but, on the contrary, are Algæ which have become imprisoned in, or invaded by, the mycelium of a Fungus, forming the thallus, in which, as it grows, the algal cells multiply by division. This led to the remarkable inference that a Lichen is a composite, not a simple, organism, consisting of Alga and Fungus living together in a relation which, on the whole, is one of mutual advantage; an altogether new biological conception, subsequently designated "symbiosis" by De Bary. This conclusion aroused the most lively opposition from the professed lichenologists. For years the famous controversy raged, conducted with much acuteness and no little acrimony, nor has it even now completely died out. It may be added that such new facts as have since been discovered, notably, the observations of Stahl (1877), have contributed to strengthen the position of the Schwendenerian theory, and that it has long been almost universally accepted.

Schwendener took no part in the controversy, nor did he publish anything more on Lichens. Whilst still at Basle, he turned his attention to a line of research more in accordance with the natural bent of his genius, the study of the mechanics of the structures of plants. The first fruit of his labours in this direction was his 'Das mechanische Prinzip im anatomischen Bau der Monokotylen' (1874), in which he demonstrated that the anatomy of these plants, and more especially the distribution in them of the supporting tissue (stereom), is in accordance with the recognised principles of constructive engineering. This line of research he pursued to the end of his career, applying mechanical principles to the elucidation of various structures and physiological processes. Before he left Basle in 1877, he published a considerable work on phyllotaxis, considered from this point of view, 'Die mechanische Theorie der Blattstellungen.' When at Berlin, he wrote a number of papers on such subjects as the twining of plants, the ascent of sap, the mechanism of the stomata and of the pulvini of leaves. Nearly all of these appeared in the 'Monatsberichte' of the Prussian Academy; they were republished in two volumes in 1898.

Not only was he active in research, but he was also successful as a teacher. He inspired a number of his students to prosecute research along the lines that he had laid down. He inaugurated, in fact, that study of structure in relation to function, which has since been so brilliantly developed, in particular by Prof. G. Haberlandt, once his pupil, now his successor, at Berlin.

Schwendener's name will always hold a distinguished place in botanical history, as that of the discoverer of the true nature of Lichens, and of the founder of the study of the physiological anatomy of plants.

The information concerning Prof. Schwendener given in this notice was kindly supplied by Prof. Dr. Hans Schinz, Director of the Botanic Garden, Zurich.

S. H. V.

Thomas R. Fraser.

SIR THOMAS RICHARD FRASER, 1841–1919.

Sir Thomas R. Fraser, M.D., F.R.S., and F.R.S.E., died at his residence in Edinburgh on January 4, 1920, in his eightieth year. Only sixteen months previously he had relinquished his Professorship of Materia Medica, in Edinburgh University, which he had held since 1877.

He was born in 1841, in India, a country to which he was destined to return, many years later, as member and President of a Plague Commission. His early education was obtained at private schools and was succeeded by his entrance as a medical student at the University of Edinburgh. As a student in that university he had come under the influence of Sir Lyon Playfair, Professor Hughes Bennet, Sir James Y. Simpson, and Sir Robert Christison, who, together with other members of the medical faculty, formed at that time a galaxy of talent probably never excelled in the history of any British medical school. It may well have been owing to such inspiring influences that many who had the advantage of studying in that era were destined to make their names worthy of a place amongst those of their renowned teachers. The most eminent toxicologist of his time, Sir Robert Christison, impressed his students by his exceptional power of observation and deduction coupled with the resources of the widest knowledge of the action of drugs, whether as poisons or as therapeutic agents; but he lived and taught before the analytical methods of pharmacology had been satisfactorily established. Thus, whilst his description of his own symptoms, subjective and objective, occasioned by a toxic drug of unascertained action (the Calabar ordeal bean) is extraordinarily exact and instructive, it was not to be at his hands that the elucidation of these effects was to be accomplished, but by the subsequent work of Fraser, who subjected the poison which had occasioned them to exhaustive pharmacological research.

This experience of Christison's, which but for his presence of mind, might have ended in disaster, occurred in 1855, and seven years later Fraser offered for graduation a thesis entitled "The Action and Uses of the Calabar Bean." He had still to devote much thought and experimentation to the subject, but on this, his first comprehensive investigation, his title to be ranked among the pioneers of pharmacology may justly be founded. Christison was not slow in recognising the early promise thus displayed by his former pupil, who became his Assistant in the University Department of Materia Medica, in the year subsequent to the appearance of the thesis. During his tenure of this position Dr. Fraser made further advances in investigating the action of drugs, especially of Calabar bean. Working largely with the extract containing the alkaloid, physostigmine (often referred to also as eserine), he recognised the altered condition of vision it occasioned, the failure of motor-cord conduction, as well as of cardiac and respiratory activity, and he indicated its application to ophthalmic practice, to the treatment of toxic spasm, and as possessing in certain intestinal conditions the value of a cathartic.

From this research there was a further development. The symptoms produced by Calabar bean and the cause of their occurrence suggested the probability that atropine, which antagonises the action of physostigmine on the eye, would also show itself antidotal with regard to the more serious effects which the former occasions. This anticipation proved fully justified by the results, and much light is shed by the long series of experiments on the relative antidotal efficiency of atropine when administered before (prophylactically), simultaneously with, or subsequently to the toxic Calabar bean extract. This work, published just fifty years ago, is a model of its kind, of care in design, and precision in accomplishment. It was also in the late "sixties" that Prof. Crum Brown and Dr. Fraser began collaboration in an experimental enquiry into the connection between chemical constitution and physiological action. Selecting certain alkaloids possessing well-defined toxic actions, amongst which spasm is conspicuous, they introduced an ethylic or methylic group into the molecule of these by substitution for hydrogen. On submitting such salts to pharmacological tests, the observers established the fact that whilst the toxicity of these was enhanced as contrasted with that of the alkaloids individually from which they were derived, the condition of spasm was no longer present, but in place a paralytic state attributable to a peripheral action on motor nerves. Confirmation with amplification of these observations were subsequently obtained in the case of atropine and coniine, of which the dimethylic iodide was found to be inferior in toxicity to the monomethylic.

The appointment of Dr. Fraser, in 1870, to an assistant physiciancy in the Royal Infirmary of Edinburgh was the beginning of a prolonged connection with that institution, as (exclusive of three years, during which he was acting as Medical Officer of Health for Mid-Cheshire) his services, first as assistant and subsequently as physician and one of the professors of clinical medicine, extended over a period of forty years.

When, in 1877, he was appointed to the Chair of Materia Medica, in succession to Sir Robert Christison, Dr. Fraser was in his thirty-seventh year. The post, with its high traditions, offered great opportunities to the enthusiastic investigator and teacher; he had, it is true, lectured "extra-murally" on his chosen subject, but he had now to meet a much larger class, to which he owed the duty of making the wide and exacting group of subjects taught from the Chair of Materia Medica* not merely intelligible but attractive. Prof. Fraser was not long in establishing his reputation as a successful and inspiring lecturer; clear, deliberate, and incisive in style, methodical and thorough in handling fact or theory, he infused vitality into what the medical student is too apt to regard as the dry bones of pharmacognosy by his descriptions of the action upon tissues and organisms of which drugs are capable, and of the therapeutic employments which are thus indicated. His lectures, no less than his devotion to research, were calculated beyond merely instructing to produce a stimulating and enduring impression upon students in the third year of their curriculum. Many of them came into contact with him again at a later period of study in the clinical wards and lecture theatre of the Infirmary. Here Prof. Fraser was in a sphere entirely congenial to him; his minute attention to detail, thoroughness of method, and wide experience rendering him a diagnostician of high order, whilst his intimate acquaintance with the potentiality of therapeutic agencies gave peculiar point to the line of treatment which he advocated.

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* The professor was responsible not only for the teaching of pharmacognosy, but also of pharmacology, therapeutics, and pharmacy.

Pharmacological research, to which he was devoted, occupied a large share of Prof. Fraser's time, and brief reference may be made here to some of the directions in which it was prosecuted. An investigation into the action of *Strophanthus hispidus* (Kombé) is closely associated with his name. The toxic properties of the plant had been known to travellers, who had ascertained that certain South African tribes used arrows, both in warfare and in the chase, which had been anointed with an extract derived from Kombé. Some of these poisoned arrow-heads, having been sent home, came into the hands of Sharpey about 1862, who recognised the extract upon the points as having the effect of a cardiac poison, whilst three years later Hilton Fagge, with Stephenson, expressed a confirmatory opinion, grouping the new poison with digitalis and squill. The further investigation which was to result in the addition of a new and valuable therapeutic agent was taken up at this point by Dr. Fraser, who, in 1870, published certain results which he had obtained with the poison from the arrow-heads; whilst, many years later (in 1885), at a meeting of the British Medical Association, held at Cardiff, he indicated the directions in which the activities of the drug might be used with advantages therapeutically.

In two extensive papers which appeared in the 'Transactions of the Royal Society of Edinburgh' in 1890 and 1892 respectively, he gives an exhaustive account of strophanthus as used as an arrow poison, discussing also its botanical features, especially the morphology of the seeds—as the source of the extract, and indicating the properties of the active glucosideal constituent, strophanthin, which he had separated from them. Whilst recognising the similarity of action which strophanthus and digitalis produce upon the heart, he emphasised the relatively feeble effect of the former upon the blood-vessels, those of the frog contracting not at all to extract solutions of 1:20,000, and but slightly to 1:1000. He also drew attention to the anæsthetic action of the glucoside on the conjunctival surface.

Many years later, another Strophanthus (*S. sarmentosus*) was investigated by him, together with Alister Mackenzie, the results published in 1911 establishing the parallel activity possessed by it with that of the *S. hispidus*.

Poisoned arrows in general had very naturally become objects of interest to him, and many specimens from widely separated sources found their way to the Edinburgh laboratories, and there were subjected to examination.

Amongst these were the weapons of the Abors and Mishmis of N.E. India, upon the points of which the toxic element was discovered to be an aconitine (probably pseudaconitine from *A. ferox*), but Sir Thomas found that the latter tribe also employed a poison which, when injected, produced hæmorrhagic conditions similar to those originated by snake venom. This poison he identified as croton oil.

He bestowed much time upon various researches into the action of snake venom, the antidotal treatment of conditions due to its absorption, and the endeavour to establish immunity by treatment anterior to venom inoculation. A series of papers embodying his results and conclusions was published in the years 1895–1897. Over a period often extending to many weeks, gradually increased doses of the venoms of certain snakes were administered, until a proportion had been arrived at which would infallibly have proved fatal to an untreated animal.

Such preparations were made by feeding,* as well as by hypodermic injection. Prof. Fraser satisfied himself that an animal thus prepared not only acquired protection itself against the action of venom subsequently administered by injection in doses largely exceeding the calculated lethal, but further, that the serum (whether liquid or desiccated) of such an animal had the property of immunising other animals against venom action. By varying the time-relationship of administration of the antivenin and of the potent venom, he sought to ascertain the limit within which the former might prove effective as a prophylactic or as a curative agent. That the bile of poisonous snakes (African and Indian cobra, puff-adder), as well as that of the non-poisonous, and of all mammals, to which the test was applied, is capable of destroying the activity of venom when a requisite portion of the former is brought into contact with the latter *in vitro*, was demonstrated by Prof. Fraser in an elaborate series of observations. Cholesterin appears to be the inactivating constituent. He entertained some hope that a practical antidotal method of treatment might find its basis on this fact, although estimating that a relatively enormous dose of bile (×1600–2000) might be required when inoculation of venom had already taken place.

The venom of several poisonous snakes was subjected to examination more recently in the Edinburgh laboratories. That of the common Krait (*Pongarus cœruleus*), investigated by Sir T. Fraser, with the collaboration of Major Elliot and Dr. Sillar, was found to be inferior in toxicity to cobra venom. Together with Dr. Gunn, the venom of the S. African Colubrine, *Sepedon hæmachates*, and also that of the Viperine, *Echis carinatus*, were examined by him, and the important conclusion was deduced from the results obtained, that the venoms of Colubrine and Viperine snakes, far from being identical, are widely different in their actions, the hæmolytic effect predominating in the latter, whilst a paralysant action on both central nervous system and motor nerve endings at the periphery is the dominating effect of the former.

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* Calmette is at issue with these conclusions in so far as the development of immunity is concerned, he (Calmette) holding that a venom-fed animal develops no antivenin—though he admits the possibility of some degree of protection being conferred upon certain very young animals by this method.

In consequence of a severe outbreak of plague in 1898, a Commission was appointed by the Governor-General, with the approval of H.M. Secretary of State for India, which was charged with conducting an enquiry into the origin of the disease, the manner of its communication, and the effects of certain sera as means of prevention or cure. As such an enquiry, from its nature, demanded the presence of the Commissioners in India, Prof. Fraser, who had been nominated for service as their President, asked and was granted leave of absence from his University duties.

Having arrived in Bombay, the Commission at once began its enquiries, examining numerous witnesses, and making searching personal observations in many of the plague-stricken areas. Much important information was elicited, which finds incorporation in the voluminous report issued in 1901. Therein also the Commissioners make suggestions as to the proceedings most likely to prove effective for preventing or circumscribing plague outbreaks in the future. These suggestions are not adopted in all instances with entire unanimity, and consequently there are presented certain minority recommendations, amongst which is an important expression of opinion by the President.

In addition to his service upon this Commission, Prof. Fraser was called upon at various times to occupy other important positions. In 1881 he acted as President of the Section of Materia Medica and Pharmacology at the International Medical Congress, which was then held in London. He filled the Presidential Chair of the College of Physicians of Edinburgh, and he was appointed by the Admiralty a member of a Committee charged with an enquiry into an outbreak of scurvy, which occurred in Sir G. Nares' Arctic Expedition. He also acted as Consulting Medical Officer to the Prison Commissioners for Scotland. He was appointed Honorary Physician to H.M. the King in Scotland in succession to Sir Wm. Gairdner. The honour of Knighthood was conferred upon him in 1902.

Apart from his professional work, Sir Thomas Fraser served his University in various capacities. His precision in method, administrative ability, and close acquaintance with the requirements of medical education, peculiarly fitted him for the discharge of the duties of Dean of the Medical Faculty, and this onerous post was held by him for twenty years. He was actively concerned in the preparation of memoranda relating to the Scottish Universities' Bill, and in facilitating the work of the Commission of 1889, which held its sittings in Edinburgh. Sir Thomas was elected by his University in 1905 as its representative to the General Medical Council, and acted in that capacity for the ten years ensuing; apart from his contributions to the main business of the Council, he rendered very special and important assistance as a member of the Pharmacopœial Committee of that body, which was then engaged in the preparation of the current issue of the 'British Pharmacopœia,' which appeared in 1914.

Wide recognition of the services which Sir Thomas Fraser had rendered to medical science and education is evidenced by the many distinctions which were bestowed upon him by learned bodies both at home and abroad.* He was created an honorary M.D. of Dublin and a B.Sc. of Cambridge. In 1877 he was elected a Fellow of the Royal Society and also of the Royal Society of Edinburgh. He was laureated by the Institute of France, by the Turin Academy of Medicine, and by the College of Physicians of Philadelphia.

In 1918, at the time of his retirement from the Chair of Materia Medica, which he had occupied for forty years, Sir Thomas Fraser received a double mark of appreciation; from his University, the honorary degree of LL.D. (a similar recognition having already been conferred upon him by Aberdeen and Glasgow), whilst, by many friends and former pupils of his own, he was presented with the replica of a portrait of himself. This portrait will perpetuate for those who knew him the harmonious sensitive features, the keen eye, and the dignified self-possession of posture with which so many generations of Edinburgh students have been familiar.

In his earlier years, if not actually robust physically, he was as alert and energetic in occupying the scanty opportunities which presented themselves for relaxation as in fulfilling the duties of the day. He had many resources for leisure. He was devoted to nature, so that gardening, which enabled him to study the growth and colour development of plants, had a strong attraction for him; hill climbing, not merely as congenial exercise, but for the pleasure of the distant view from the summit, led him at one time or other to make the ascent of all the highest mountains in Britain. Shooting, but more especially trout fishing, in which he was an adept, were sports which he followed with enthusiasm. He recognised the need of outdoor exercise for others, who, like himself, were spending the bulk of their time in class-rooms, laboratories, and infirmary wards, by encouraging rowing amongst the undergraduates, of whose boat club he was for many years the captain.

The sense of pleasure and refreshment, which in more advanced life Sir Thomas experienced when a vacation permitted him to visit his Highland residence, "Druimbeg," in Argyleshire, can be readily appreciated. There, freed for a time from the close succession of professional duties, living amidst beautiful surroundings and in the companionship of his wife and family, he could supervise his garden—the many medicinal plants in which were of special interest to him, and enjoy the congenial opportunities for recreation which were readily available.

But for many years before the end of his active life his health had not been satisfactory. He suffered from a bronchial condition, which was sometimes incapacitating, and must often have rendered the discharge of

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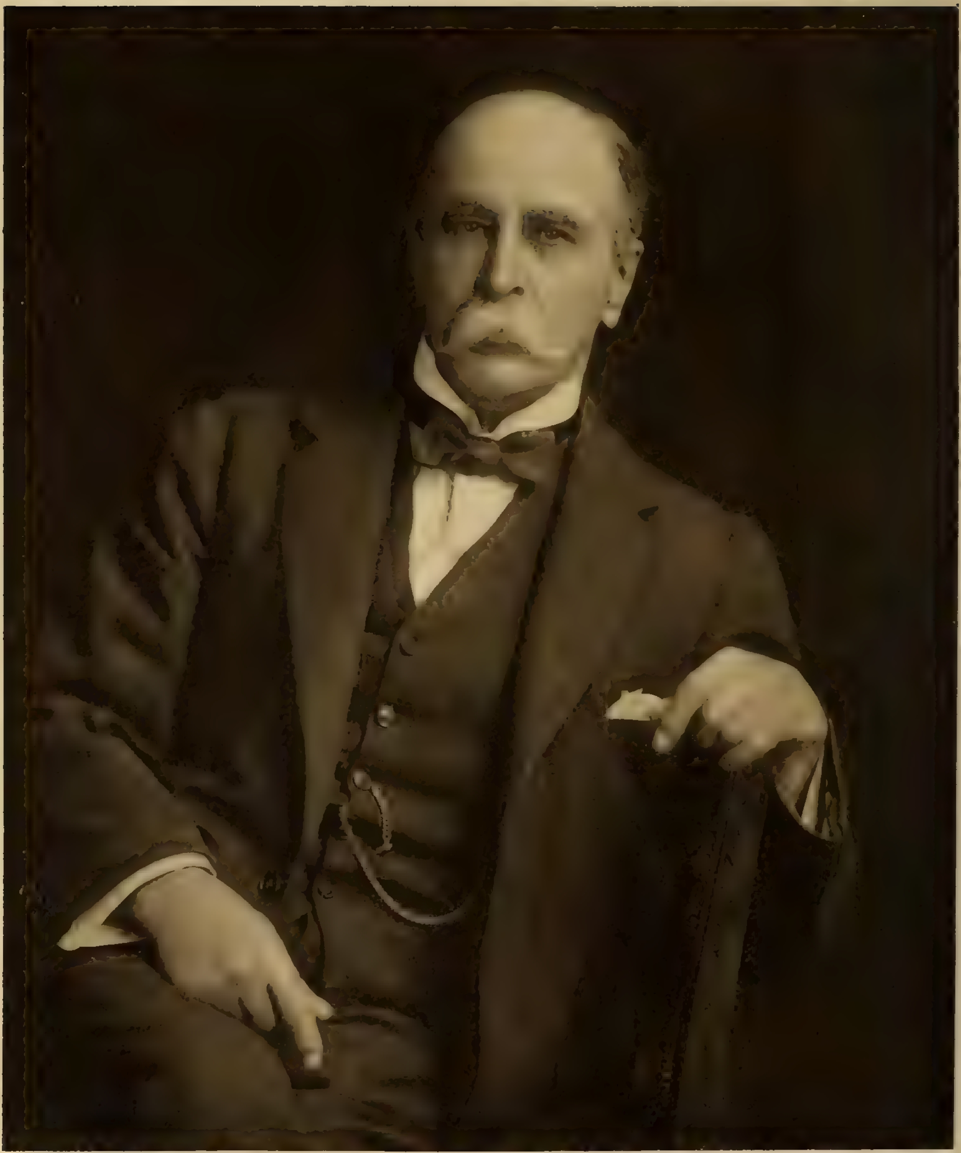
* The substance is given here of a paragraph contained in an excellent appreciation of Sir T. Fraser which appeared in the 'Scotsman' newspaper on the day following his decease.



Elliott & Fry photographers [illegible]

Wm Osler

duties which involved speaking to large audiences trying to him. When he had reached the age of seventy he had a further misfortune, in experiencing a fracture of the femur, and, though partial use of the injured limb was eventually recovered, his movements remained restricted, and were often attended by discomfort. In spite of these limitations, Sir Thomas never lost his courageous spirit, which supported him in carrying on the performance of his duties and attaining the object he had in view, although in this attempt he might have failed had it not been for the constant and devoted care which Lady Fraser exercised on his behalf. His interest in research work never forsook him, for even after his retirement, and within not many months of his decease, he was planning fresh investigations, of which he was not to see the accomplishment. As his mind remained active and vigorous to the end of life, so in his appearance there was little indication of the advanced age to which he had attained, for his hair was still moderately dark and abundant, whilst his features retained not only the alertness, but much of the symmetry of earlier years.

J. T. C.

SIR WILLIAM OSLER, Bart., 1849–1919.

It is no easy task to write an obituary notice of Sir William Osler which shall be in any degree adequate. No one who did not know him could give a just appreciation of the man, and those who did know him retain so dominant a memory of his vivid personality and charm that they are apt to do less than justice to his achievement, and to those strenuous years of scientific work which earned for him the opportunity of manifesting the gifts which were so pre-eminently his.

The story of Osler's life falls naturally into three main periods. The first thirty-five years were spent in Canada, where he was born and educated, and, after his graduation followed by two years' work in Europe, were devoted to steady work in the study and teaching of physiology and morbid anatomy and the pursuit of clinical medicine. Then followed twenty years in the United States. This also was a period of research and observation, during which he organised the medical teaching of an important new university, and trained a group of able younger men, who were destined to carry on his work and to hand down its traditions.

The last fifteen years, spent in Oxford, constituted a period of fruition, of the cultivation of many interests, and of widespread influence upon the advancement of medicine and of medical education. Success seemed to come to him without his seeking it, and his was the rare distinction of having occupied professorial chairs in four universities, in three English-speaking countries, each one of which he was invited to fill.

His father, the Rev. Featherstone Osler, a clergyman of the Church of England, emigrated, with his wife, in the year 1837, from Cornwall to Canada, to take up missionary work in that country, and settled in the province of Ontario. There, in 1849, at Bond Head, the subject of this memoir was born, the eighth of a family of nine children, several members of which have attained to positions of distinction. His father, who died in 1895, and his mother, who reached the patriarchal age of a hundred years, lived to see and to rejoice in the successes of their sons. When the boy was nine years old his parents removed to the more settled district of Dundas Valley, where was a good grammar school. At his second school, at Weston, near Toronto, Osler came under the influence of one of those who helped to shape his career, the Rev. W. A. Johnson, the head master, a man keenly interested in natural science and who possessed a microscope. He it was who first awakened in his responsive pupil a zeal for microscopic work and those scientific interests which played so large a part in shaping his later life.

Probably each one of us cherishes the memory of such teachers who influenced us strongly, and although these guiding personalities of our early days tend to assume heroic proportions with the lapse of years, the parts which they play in our lives admit of no question. Osler was fond of recalling three such men, of whom Johnson was one, and to that trio he dedicated his text-book of medicine.

Influenced by his upbringing, Osler contemplated following in his father's footsteps and taking orders. With that intention he entered at the Trinity College, Toronto, where he came under the second of the three teachers, James Bovell, Professor of the Institutes of Medicine, a physician who himself afterwards took orders. Having relinquished the idea of entering the Church, Osler took up the study of medicine, his true vocation, and, after two years spent at the University of Toronto, migrated to McGill University, Montreal, where he completed his medical course, and graduated in 1872.

At McGill he was a pupil of Dr. Palmer Howard, the Professor of Medicine, the third of his outstanding teachers, who probably influenced him most of all.

After taking his degree he proceeded to Europe, and worked in London, at University College, in Berlin and Vienna, under a number of distinguished men, including Jenner, Wilson Fox, Ringer, Virchow, and Nothnagel. The experience thus gained in British and Continental methods of medical education and research stood him in good stead when he was called upon to organize the medical clinic at the Johns Hopkins University.

In 1874 Osler returned to Montreal, to take up the Professorship of the Institutes of Medicine at McGill, at the early age of twenty-five.

The ten years during which he held that Chair were years of active progress of the school, and in that progress he had no small part.

The subjects of his professorial lectures were physiology and pathology, and he also accepted the Chair of Helminthology at the Veterinary College, but ere long he was given charge of a small-pox ward, and in due course was elected a physician to the Montreal General Hospital. Thus he acquired opportunities of clinical teaching, of which he took full advantage, and, ten years after his return to Canada, had gained such a reputation as a physician and teacher of clinical medicine that he was invited to accept the Chair of Medicine in the University of Pennsylvania.

It is evident, from the testimony of his colleagues and pupils, that the influence and stimulating power, which were so characteristic of Osler throughout his life, were fully in evidence during the Montreal period. The ties there knit were never loosened; it was to his *alma mater*, McGill, that he bequeathed his valuable library, and he desired that his ashes should rest within her walls, amidst the books which he loved so well.

His sojourn in Philadelphia lasted only five years. They were years of strenuous work and growing fame as a clinical worker and teacher who always maintained the tradition that to be a good physician a man must needs be a well-trained pathologist. Of Osler, in Philadelphia, Dr. Howard A. Kelly writes:—"Fresh invigorating currents of life and new activities in our stereotyped medical teachings began to manifest themselves, and every sturdy expectant youngster, in short order, lined himself up as a satellite of the new star. Osler breezes were felt everywhere in the old conservative medical centre, and yet it was not without some difficulties that he securely established himself."

In 1889 he accepted the call to take up the posts of Professor of Medicine in the newly established Johns Hopkins University in Baltimore and of Physician-in-Chief to the Johns Hopkins Hospital. The new University had acquired an ideal President in Dr. Daniel C. Gilman, and a group of most able professors. Amongst those who formed the nucleus of a medical faculty were Drs. Welsh, Newell Martin, and Ira Remsen. The other clinical chairs were filled by the appointment of Drs. Halstead and Kelly. Prof. Welsh in the Chair of Pathology was a tower of strength, and the new Professor of Medicine was most fortunate in his colleagues.

At Johns Hopkins Osler found his great opportunity. He could construct a medical clinic *de novo*, unhampered by traditions or vested interests. It was for him to determine the staff required, to select its members, and to shape the methods of teaching and research; and under his guidance there emerged the earliest organised medical unit in any Anglo-Saxon country.

In its construction he adhered firmly to the best and essential feature of the English schools, the contact of the student with the patients, in the wards and out-patient departments, throughout his curriculum, and the teaching of medicine at the bed-side. At the same time he embodied what is best in the German system, the intimate association of laboratories and wards, under one directing head, and the enlistment of a group of highly trained assistants working under the guidance of the director. The advancement of the science and art of medicine was one of the chief objects aimed at, and even the clinical clerks were taken into partnership for this work, were encouraged to collate cases, to look up references and historical points, and were trained in exposition.

It was only gradually that the structure grew. First the clinical and pathological scheme was constructed, whilst as yet there were no students to profit by it. Then followed a period of post-graduate teaching only, and when, in the end, the first undergraduate students reached the wards, they found awaiting them a clinic fully organised and equipped.

From the great school so built up many others have since been copied or developed, and it is not too much to say that from Johns Hopkins has emanated an influence which has revolutionised the whole system of medical education in America; nor has its influence failed to spread to our own country. Many of Osler's assistants and pupils occupy leading posts in other universities, and the profession pays ungrudging tribute to the work which he accomplished for medical teaching and research. Even if the work which Osler did at Johns Hopkins stood alone to his credit, it would ensure for him an honoured place in the annals of medicine.

Nor were his activities limited to the university, the whole of the medical life of Baltimore and Maryland felt his influence. He infused new life into societies and libraries, and took a leading part in the work of combating tuberculosis in the State, and in the promotion of sanitation. Throughout the length and breadth of the United States his influence was felt, working always for co-ordination of the medical profession and the brotherhood of its members. It was for him a time of literary activity also; and in 1891 his text-book of medicine was published. In England his work and eminence were fully recognised. He had been elected to the Fellowship of the Royal College of Physicians in 1884, whilst still in Montreal, and delivered the Gulstonian Lectures in the following year. In 1898 he was admitted to the Fellowship of the Royal Society.

In 1892 he married Grace Revere Gross, daughter of John Revere of Boston.

The work at Johns Hopkins, coupled with the claims of a large consulting practice which knew no limits of mileage, was very exacting, and after other invitations had been declined, at various times, he accepted, in 1904 the office of Regius Professor of Medicine in the University of Oxford, on the resignation of Sir John Burdon Sanderson.

In Oxford, whither he came in 1905, Osler entered upon a third period of activity, as the occupant of a Chair dating from the reign of King Henry VIII and with very different functions from those of his previous Chairs; a position of high prestige, which had been enhanced by the tenures of Acland and Sanderson, with great opportunities of influence but little clinical teaching. The duties of the Regius Professor are varied, and others are added to them. Osler soon came to his own and held an unrivalled position in Oxford. He took an active part in the administrative work of the University and its medical school, of the Bodleian Library as a Curator, and of the Clarendon Press as a delegate. Coming fresh from a land of intensive progress he was able to suggest new methods and improvements. At the Radcliffe Infirmary he had opportunities of clinical teaching, kept in touch with medicine, and took an active part in promoting the development of the Pathological Department of the hospital. King James I attached to the Regius Chair the Mastership of the ancient fifteenth century almshouse at Ewelme, and Osler took an intense interest in its beautiful buildings, and its precious muniments, of which he secured the proper care.

Osler rejoiced in the atmosphere of an ancient seat of learning, with its long traditions of men and movements. Before his arrival, he had been elected to a studentship at Christ Church, the college of two of his literary heroes, Burton, author of the 'Anatomy of Melancholy,' and John Locke. One could not help feeling that, whereas his mind was in the forefront of all progress, and his energies were devoted to the interests of the future, he felt equally at home in the past, and found a congenial setting in the Middle Ages.

There were few of those amongst whom he moved who did not come under the spell of his friendship. Recognised as a great physician, a man of science, an eminent teacher of medicine and student of its history, a learned lover of books for their contents rather than their outward form, and a power in educational matters, he easily held his own in any gathering of learned men, and his advice and opinion were valued highly. No greater tribute could have been paid to his versatility than his election, in the last year of his life, to the Presidency of the Classical Association.

He had accumulated a great library of books bearing upon medicine from its earliest days, which contained many rare works, and almost every known edition of his life-long favourite, the 'Religio Medici' of Sir Thomas Browne. He had made considerable progress in its classification and indexing on a novel and original plan, and few things gave him greater pleasure than to show and describe his treasures to an appreciative listener.

A facile orator, Osler could make an apt and interesting speech on any occasion. He could not be tedious, and his speeches, as also his writings, were studded with phrases which fixed themselves in the memory of his hearers, and were permeated by an impish humour which was essentially his own.

Sometimes, indeed, his lighter sayings were misunderstood, and a statement, or rather over-statement, thrown off with a smile in an after-dinner speech, was, on occasion, made the subject of a grave discussion in the daily press.

His house in Oxford was a centre of wide hospitality, wherein he and Lady Osler extended a cordial welcome to their many friends. It was a place of pilgrimage to Americans and Canadians who came to England, and its doors were ever open to the Transatlantic Rhodes scholars.

Nor were his activities limited to Oxford. He was often in London, at the Royal College of Physicians, the medical societies and schools, and at the many important committees of which he was made a member. He took an active part in the foundation of the Historical Section of the Royal Society of Medicine, and of the Fellowship of Medicine, started during the war to facilitate post-graduate work for officers and colleagues from overseas. He presided over the Medical Section of the International Medical Congress at its meeting in London in 1913, and it was obvious that members from all lands, who attended the Congress, gathered round him not merely as appreciative colleagues but as personal friends. Soon after he came to Oxford, Osler suggested the formation of the Association of Physicians of Great Britain and Ireland, a body of clinical teachers which has done much for the advancement of scientific medicine, and in bringing together the staffs of the various medical schools of these kingdoms. He also was one of the founders, and until his death the senior editor, of the 'Quarterly Journal of Medicine.'

Throughout his career Osler made many contributions to medical literature. His earliest published writings were the outcome of his microscopic work, and dealt with Canadian Diatomaceæ and the platelets of the blood, which he was one of the first to describe. The first to observe them was Max Schultze in 1865. Osler showed, in a paper published in the 'Proceedings of the Royal Society,' in 1874, that the clusters which Schultze had described were formed by the aggregation of separate particles which circulated as such in the blood. Some ten years later, Bizzozero gave them the name of platelets, and indicated the part which they play in thrombus formation.

In 1877 Osler described a verminous bronchitis occurring in dogs, and the causative organism which is sometimes spoken of as *Filaria Osleri*. He fell into the error, however, of classifying the organism as a strongylus, whereas it is a filaria.

Many of his papers are records of individual cases; the earlier ones are mainly pathological, but, after a time, clinical papers are included, and, in the end, predominate. They reflect the subjects which were occupying his attention from time to time, such as the prodromal rashes of small-pox, miners' lung, the changes in the blood in disease, and the forms of splenic enlargement. Not a few papers treat of arterial diseases, of aneurysm, and malignant endocarditis, which last he chose as the subject of his Gulstonian Lectures, delivered at the Royal College of Physicians in 1885.

The clinical papers cover a large part of the field of medicine. Tuberculosis, syphilis, chorea, the cerebral palsies of children, congenital heart disease, pulmonary fibrosis, congenital malformations, ochronosis, gastric and duodenal ulcers, typhoid fever and its complications, malaria and cerebro-spinal fever are among the subjects of which they treat. Some subjects recur at intervals, such as the visceral lesions of the erythema group. Vacquez first described a case of polycythæmia rubra, but it was Osler who recognised it as a definite clinical entity, and it is often connected with his name. An hereditary malady, characterised by multiple telangiectases associated with hæmorrhages, may rightly be styled Osler's disease. The knowledge embodied in these papers, which cover so wide a range, formed an admirable ground-work for a great text-book, and his Text Book of Medicine was Osler's *magnum opus.* In it he made a judicious use of statistics collected from hospital cases; it gains much from the inclusion of tables and charts, and he made full use of the researches of others. The clear and individual style of the book makes it very helpful to students and practitioners alike, and it has long held the foremost place among such works, both in this country and in America. One great feature is the due weight given to morbid anatomy. The book has passed through many editions, and has been translated into French, German, and Chinese. The ninth edition, upon which he was engaged, has appeared since Osler's death, under the editorship of Dr. T. MacCrea, with whom he had previously edited a System of Medicine.

Valuable as Osler's researches were they cannot be described as epoch-making. What was epoch-making was the work which he instigated and inspired; the stimulating influence which he exercised upon his assistants who worked under his guidance, upon the hundreds of students whom he taught, and upon the thousands who have read his text-book and other writings. He had the gift of being genuinely interested in the work of all his pupils and friends, and spared no pains to help even the least of these in what he was trying to do. From wherever Osler might be there issued telegrams, letters and post-cards, conveying here a word of sympathy or congratulation, there a pat on the back to the writer of a paper which pleased him, or a note on something which he thought would be helpful to a fellow worker.

His scientific writings by no means exhausted his literary activities. Essays and addresses of compelling interest and admirable in literary style, such as were collected into the volumes with the titles of "Aequanimitas" and "An Alabama Student," lay sermons, such as "A Way of Life" delivered to the students of Yale, and introductions to reprints of old books, appeal to a wider audience, and give us a revelation of his thoughts and ideals.

Many honours came to him. Many universities conferred upon him their honorary degrees, and the Academie de Médecine of Paris elected him as a Foreign Associate. He was made a Baronet in 1911, at the time of the King's Coronation. One tribute which gave him special pleasure was the presentation, on the eve of his seventieth birthday, of two volumes of essays written in his honour by pupils and friends upon both sides of the Atlantic. The presentation was made, in London, by his brother Regius Professor of Cambridge, Sir Clifford Allbutt, in a speech worthy of the occasion, which elicited from Osler an equally felicitous reply, spoken with deep emotion and lacking none of his old charm of diction.

The last years of Sir William Osler's life were spent under the shadow of the great war. He felt the incubus deeply, but spared no effort to help. Long railway journeys, under conditions trying to a man of his age, attendance on many committees, inspections of many hospitals, and especially those conducted under American and Canadian auspices, laid a heavy burden upon him. In 1917 he and Lady Osler suffered the loss of their son, their only child, who fell in Flanders when serving with the artillery. Yet deeply bruised as he was, he carried on bravely, as ready as ever to help others and working as hard as ever. His own loss served only to increase his affectionate sympathy for his friends in like case.

Early in October, 1919, he fell ill, after a long, cold motor drive from the north, where he was held up by the railway strike, and to the illness so acquired he succumbed some three months later, on December 29th, 1919, leaving a gap which cannot be filled, and the memory of a mind and character hardly to be matched in their compelling influence, versatility and charm.

A. E. G.

JOHN GILBERT BAKER, 1834–1920.

John Gilbert Baker was born at Guisborough in Cleveland, Yorkshire, on January 13, 1834. In August of that year his parents, John Baker and his wife, Mary Gilbert, removed to Thirsk, where young Baker spent his early boyhood. In 1843 he was sent to the Friends' School at Ackworth, where he evinced those interests that were to dominate his life by commencing to make a collection of local native plants. In 1846, at the age of twelve, Baker was transferred to the Friends' School at Bootham, York, which had already acquired a considerable reputation for its encouragement of natural study, a vigorous school natural history society having been in existence there since 1836. When in his fourteenth year, Baker was awarded for his collection of botanical specimens the annual prize at the exhibition of "out of school" work for 1847, and was appointed curator of the school herbarium.

Later in 1847, Baker left school in order to assist his father, and for the next eighteen years was engaged in business at Thirsk. But this occupation did not abate the predilection for natural studies developed at Ackworth and disciplined at Bootham. He collected critically, and we find him, at fifteen, contributing to the 'Phytologist,' in 1849, a new record of a rare *Carex* from Snailesworth Dale. By the time he was twenty, his knowledge of the plants of his county enabled him to collaborate with J. Nowell, who dealt with the mosses, in issuing a supplement to the 'Flora of Yorkshire,' published by H. Baines in 1840. This local supplement of 1854 was followed in 1855 by a thoughtful discussion of the relationship of the flowering plants and ferns of Great Britain to their surroundings, and by 1859 his reputation as an authority on British plants was so fully established that he then became the curator and secretary of the still active Botanical Exchange Club. Among those by whom the extent of Baker's knowledge was already fully appreciated was Daniel Oliver, a distinguished young Northumbrian botanist, four years Baker's senior, who, in 1858, had been invited by Sir William Hooker to accept the post of librarian at the Royal Gardens, Kew. The ability with which, from 1859 to 1865, Baker distributed the collections of the Exchange Club and drew up its reports enhanced the respect with which he was already regarded, and his reputation as a philosophical natural historian was placed on a permanent basis by the publication, in 1863, of a scholarly series of studies of the botany, geology, climate, and physical geography of North Yorkshire. The work which his connection with the Exchange Club entailed led Baker now to turn his attention to taxonomic problems. He published in the 'Naturalist' for 1864 a review of the British roses, which attracted immediate attention on the Continent as well as in this country. In 1865 he contributed to the 'Journal of Botany' a monograph of the British mints.

In May, 1864, Baker met with a misfortune which was to determine his future career. His work on North Yorkshire, though published in London, had been printed at Thirsk, where the bulk of the stock was stored in his business premises. A disastrous fire destroyed this stock and at the same time consumed his private herbarium and his botanical library. The members of the Exchange Club and other friends at once proceeded spontaneously to replace his lost library and did all that they could to make good the valuable botanical collection. They could not restore the stock of copies of the volume on North Yorkshire. But practical sympathy induced some at least of his friends to suggest to Baker that his misfortune would be a gain to the cause of natural knowledge were he to take the opportunity it offered him of abandoning business pursuits and of devoting himself exclusively to those scientific studies he was so well qualified to prosecute.

There is no reason to doubt that this wise suggestion attracted Baker, though when it was made its realisation must have appeared somewhat hopeless. Baker had given "hostages to fortune." In August, 1860, he had married Hannah Unthank, and their first-born child, Edmund, born on February 9, 1864, was an infant when the fire took place. A chapter of accidents, however, enabled the suggestion to be adopted.

In 1862 the widow of the accomplished W. Borrer had presented to Kew the whole of her husband's fine herbarium. The incorporation in the general collection of Borrer's vascular cryptogams was a matter of urgency in connection with work on which the Director of Kew was personally engaged. The excellence of Baker's treatment of the roses had arrested Sir William Hooker's attention. It was known, too, that in connection with his Exchange Club work Baker was then making a special study of ferns. He was accordingly invited to assist in the arrangement of the Borrer material preparatory to the laying-in of the sheets. In August, 1865, the veteran Sir William Hooker died in his eighty-first year. Among the many tasks undertaken by that eminent botanist, one of the most arduous had been the preparation of his 'Species Filicum,' the five volumes of which had occupied much of his attention between 1846 and 1864. On the completion of this great work, its author, unwearied by the weight of fourscore years, set himself the task of preparing a 'Synopsis Filicum,' and it was in connection with this new undertaking that the help of Baker in dealing with Borrer's specimens had been sought. Sir William's son and successor, Dr. (afterwards Sir Joseph) Hooker found on the Director's desk the preface to the projected 'Synopsis,' much of its matter in manuscript, and proof copies of the three opening sheets. It was clearly desirable that a work so important should be completed. The multifarious duties and undertakings of the new Director equally clearly precluded him from attempting the task. His experience of Baker's work led the younger Hooker to decide that Baker was singularly qualified to accomplish the work. The task, however, could only be carried out at Kew. This involved the creation of a post in the establishment which Baker could fill. Fortunately, it was possible to arrange that this be done. In 1861, Oliver, the librarian at Kew, had been permitted to supplement the exiguous stipend which public opinion then regarded as an adequate remuneration for scientific service, by accepting the chair of botany at University College, and in 1864, when A. Black, the able keeper of the herbarium at Kew, was compelled, owing to the state of his health, to resign that appointment, Sir William Hooker, with a view to further improvement in Oliver's position, succeeded in obtaining the approval of Government for a proposal to amalgamate the keepership of the herbarium with the librarian's post. This reduction in the strength of the establishment at Kew in 1864 was followed by a further reduction in 1865, owing to the decision that the post of Assistant Director, formerly held by Dr. Hooker, must lapse with his appointment to the directorship. Under the circumstances, however, assent was given to the proposal of the new Director for the creation of a new post, that of first assistant in the herbarium, as from April 1, 1866, and permission was accorded him to engage temporary assistance at once. On receipt of this authority Hooker invited Baker to join the Kew staff. With this invitation Baker complied, taking up his duties as a temporary officer in January, 1866, and being permanently confirmed in the post of first assistant in the herbarium on April 1 of that year.

Soon after his appointment at Kew, Baker was permitted to follow the example of his colleague in the herbarium, in supplementing his income by undertaking teaching work. In 1869 he was appointed lecturer on botany at the London Hospital Medical School, and held this post until 1881. In 1874 he was appointed one of the lecturers to the young gardeners employed at Kew, when the courses of instruction, voluntarily initiated by Oliver in 1859, at last received the recognition of Government. In 1882 he was appointed by the Society of Apothecaries to their lectureship on botany at the Chelsea Physic Garden.

Baker occupied the position of first assistant in the Kew herbarium until, on Oliver's retirement at the age of sixty, he was promoted on June 1, 1890, to the keepership of the library and the herbarium. As keeper he served until his own retirement, at the age of sixty-five, in 1899. The Chelsea lectureship he retained, after his promotion at Kew, until 1896. The Kew lectureship he consented to retain, greatly to the advantage of his pupils, for five years after his retirement from the keepership, and his demission of this work at the age of seventy in 1904 was a source of unmixed regret. But he still continued his private studies in the herbarium, and, although towards the close of his life his physical strength gradually declined, there was no impairment of his intellectual vigour. He died at Kew, in his eighty-seventh year, on August 16, 1920.

Baker's first task on reaching Kew was the completion of the 'Synopsis Filicum.' This he prosecuted with such industry that the work was published in 1868, and with such ability that he became immediately a leading authority on vascular cryptogams, and was at once invited to prepare the volume in the great 'Flora Brasiliensis,' edited by Von Martius, which deals with the ferns of Brazil. This volume, which appeared in 1870, was but one of many further contributions on the same subject. A new edition of the 'Synopsis' was soon called for, and the work was carefully revised by Baker before its re-issue in 1874. In 1875 he presented to the Royal Irish Academy an account of the ferns of the Seychelles. Sir William Hooker, in 1854, had supplemented the 'Species Filicum' by devoting the tenth volume of the 'Icones Plantarum' to the illustration of a century of new, rare, and imperfectly known ferns. Following this example, Baker supplemented the 'Synopsis Filicum' by completing in 1887 a second century of new and rare ferns, illustrated in the seventeenth volume of the 'Icones.' In 1887, also, he provided, as a companion to the 'Synopsis,' a welcome handbook of the fern-allies, and in 1892 he contributed to the 'Annals of Botany' a summary of the ferns discovered or described since 1874.

Baker's interest in roses was equally sustained. The review of the British species, published in 1864, was followed by a monograph of British roses, published by the Linnean Society in 1869, and by a revised classification of the genus, contributed to the same society in 1902. Baker also wrote the botanical descriptions of the species figured by A. Parsons in the fine monograph of the genus *Rosa*, published by Miss E. A. Willmott between 1910 and 1914.

These, however, were not the only monographic interests that Baker displayed. He became the leading authority of his day on a number of monocotyledonous natural families of plants. In dealing with this important subject, he adopted simultaneously two radically distinct methods, both of which he employed with equal success. Between 1869 and 1899 he contributed to the 'Gardeners' Chronicle' numerous accounts of monocotyledonous genera, designed especially for the benefit of those engaged in cultivation. To the 'Journal of the Royal Horticultural Society' he supplied other contributions of the same kind. As mindful of the needs of scientific workers as he was of practical requirements, he contributed numerous similar papers to the 'Journal of Botany' during the same period. Between 1870 and 1880 he communicated to the Linnean Society instalments of a monograph of the *Liliaceæ*. In 1878 the same Society published his monograph of the *Hypoxidaceæ*, and in 1887 its Journal included his 'Systema

Iridacearum.' In 1893 he contributed to the 'Annals of Botany' a synopsis of the *Museæ.* So highly were these various contributions valued, that Baker was begged to collate and systematise many of his articles in the 'Gardeners' Chronicle' and the 'Journal of Botany,' and re-issue the information in a series of valuable handbooks. One of these, dealing with the *Amaryllideæ* as a whole, appeared in 1885; another, dealing with the *Bromeliaceæ,* was published in 1889; a third, dealing with the *Irideæ,* was issued in 1892.

But Baker did not confine his attention exclusively to monocotyledons and vascular cryptogams. He was the author of numerous similar papers dealing with dicotyledonous plants. One of the most important of these, owing to its bearing on economic problems, was a monograph of the tuber-bearing species of *Solanum,* published by the Linnean Society in 1884.

Before Baker undertook to describe the ferns of Brazil, he had already displayed his aptitude for floristic, as contrasted with monographic study, in the 'New Flora of Northumberland and Durham,' prepared in collaboration with G. R. Tate, and published in 1866. The appearance in 1870 of the volume relating to ferns immediately led to an invitation to elaborate the Brazilian *Compositæ.* The results occupy two volumes, issued by instalments between 1873 and 1884, in the great work of Von Martius. But Baker had already elaborated one natural family for the 'Flora of Tropical Africa' in the first volume, issued in 1868, and another in the second volume, which appeared in 1871. He described the species of twenty-one additional families for the parts of this work published between 1898 and 1906. He also elaborated for the 'Flora of British India' an account of one natural family, published in 1876 and 1878, and of a second family, published in 1890 and 1892. In 1877 appeared his 'Flora of Mauritius and the Seychelles.' In 1885 he published his well-known 'Flora of the English Lake District.' Between 1877 and 1895 he contributed, partly to the 'Journal of Botany,' partly to the Linnean Society, a series of important floristic studies connected with the vegetation of Madagascar, which embody descriptions of over a thousand species previously unknown. He also described the species of five natural families, included in the sixth volume of the 'Flora Capensis,' published during 1896 and 1897.

Notwithstanding the extent and the excellence of Baker's systematic contributions, his interest was not wholly confined to classification. His earliest note, published in 1849, is concerned with the environment rather than with the characters of the plant to which it refers. In the striking paper published by him in 1855, when he had barely attained his majority, Baker attempts to classify British plants "according to their geognostic relations." His work on North Yorkshire, which was published in 1863, is a natural history of the area discussed, of such value that the Yorkshire Naturalists' Union during the period 1888–1906 accorded it the unusual honour of republication in their Transactions in a revised form. Baker took the opportunity afforded him by the preparation of the 'Synopsis Filicum' to draw up a complementary review of the geographical distribution of ferns which was published by the Linnean Society in 1867. Numerous other papers dealing with questions of distribution were published by him in later years and at the close of his first course of lectures at Kew in 1874 he was invited to contribute to the 'Gardeners' Chronicle' a series of "Elementary Lessons in Botanical Geography" in order "that gardeners and other learners in biology should be encouraged as much as possible to acquire comprehensive and correct ideas of the laws and leading facts of plant distribution." These articles were so greatly appreciated that he was persuaded to permit their publication in 1875 in book form. In 1888 he was jointly responsible with W. W. Newboult for a revised edition of the 'Topographical Botany' by H. C. Watson, to whom the original edition of his own 'North Yorkshire' was dedicated. This natural history interest and instinct, manifest even in those writings which duty compelled him to cast in a taxonomic mould, remained powerful to the end, for the last of his published papers, which appeared in 1917, deals with the botany and the physical geography of Palestine.

Baker's work as a naturalist and systematist received general recognition. In September, 1864, as soon as his review of the British roses appeared, Baker was elected an Associate Member of the Société Royale de Botanique at Brussels. In April, 1866, he was elected a Fellow of the Linnean Society in whose welfare he took a keen interest. He served as a member of Council during 1876–78, 1889–91 and 1893–96, acting as a Vice-president during 1889–91 and again in 1893–94. His work for the 'Flora Brasiliensis' led to his election to the Leopoldinisch-Carolinische Akademie der Naturforscher of Halle. From 1868 to 1886 he served on the scientific committee of the Royal Horticultural Society and in the last mentioned year was appointed a Vice-chairman of the Narcissus Committee, although not then a Fellow of the Society. In June, 1878, he was elected a Fellow of the Royal Society and served on the Council in 1883–84. He became a member of the Yorkshire Naturalists' Union in 1883 and was elected President for 1884–85. In February, 1886, he was elected an Honorary Member of the Literary and Philosophical Society of Manchester. After having been deprived of his help for two years, owing to modification of their bye-laws, the Council of the Royal Horticultural Society in December, 1888, did him the signal honour of appointing him an Honorary Life-fellow, thus enabling them to appoint him to preside over the botanical section of the Rose Conference of 1889 and to benefit again from that year onwards by his services as a member of the scientific committee. In 1890 the Natural History Society of Dumfries and that of Northumberland and Durham made him one of their Honorary Members. In November, 1896, he was elected a British Honorary Fellow of the Botanical Society of Edinburgh. He became a Corresponding Member of the Massachusetts Horticultural Society in July, 1898, and of the New York Academy of Sciences in February, 1899. In 1902 he was elected an Honorary Member of the Royal Irish Academy.

In 1897 the Royal Horticultural Society bestowed on Baker the Victoria Medal of Honour in Horticulture, and in 1899 he was the recipient of the Linnean Medal, the highest honour it is in the power of the Linnean Society to offer. A portrait of Baker, by J. W. Forster, was exhibited at the Royal Academy in 1893.

The service to the State which Baker was able to render as a teacher was marked. The opportunity of imparting useful botanical knowledge to so many men destined to apply it in outlying parts of the Empire has been given to few; no teacher ever took fuller advantage of his opportunity. Recognition of this aspect of his labours came late; it was in 1919 that the University of Leeds conferred on Baker the Honorary Degree of D.Sc.

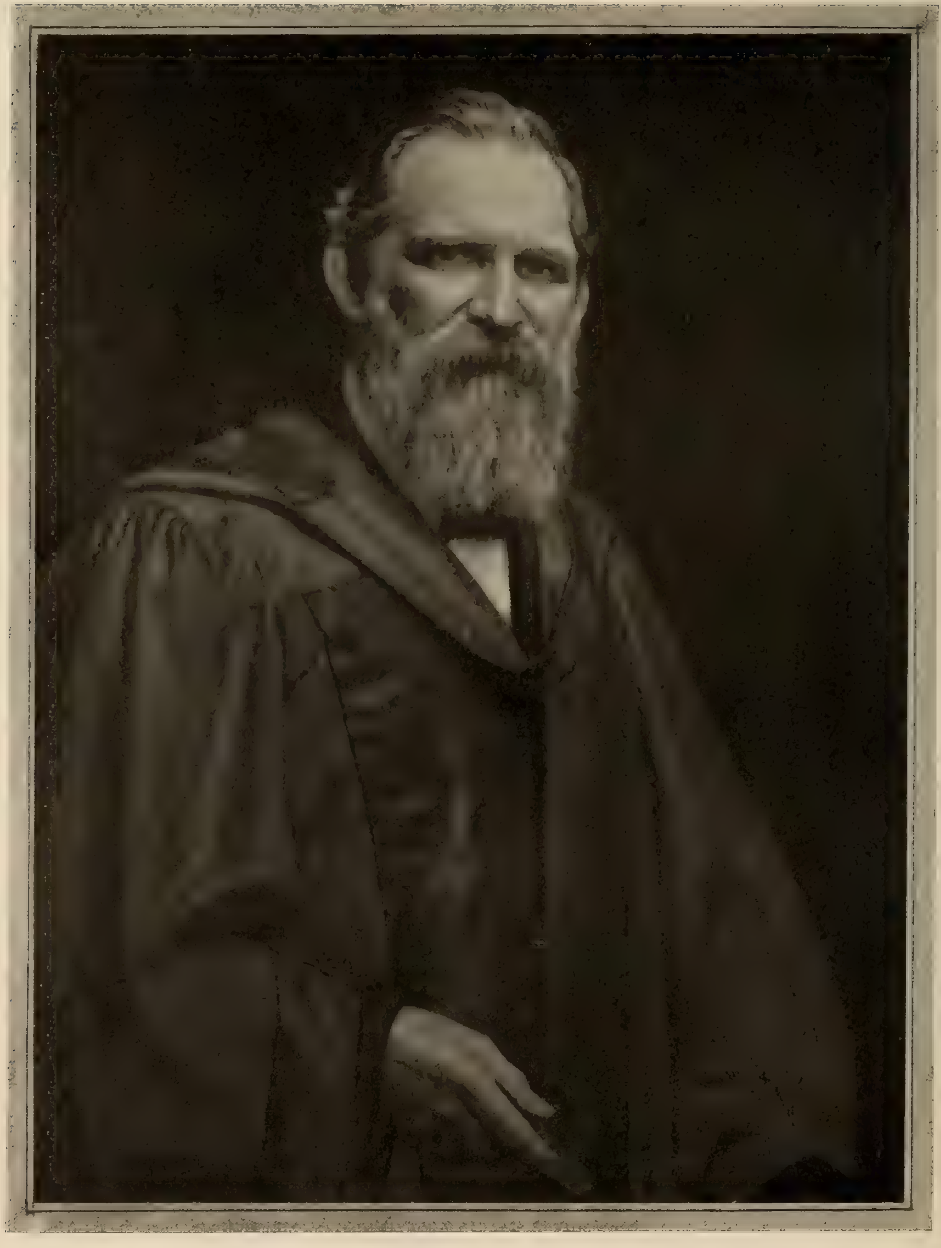
Baker owed his eminence as a systematist largely to the circumstance that his floristic and monographic studies alike are imbued with the spirit of the philosophical natural historian impelled by a sense of duty to attack taxonomic problems. The tasks he undertook clearly gave him the utmost pleasure; the spirit in which they were approached saved him from the error of regarding classification as an end. The identity of the subject of study having been established, his further interest lay in its relationship to its surroundings. He had fully apprehended the effects of environment before Œcology became a special study.

He appreciated as clearly the distinction between floristic and monographic study. He realised that the object of floristic work is to facilitate the identification of the plants that characterise a given geographical area, whereas the purpose of monographic work is to determine the affinities of the plants that constitute a particular natural group. His floristic diagnoses, so drafted as to be employed by the uninitiated in identifying with some certainty the plants in which business may cause them to take a practical interest, are divested of those details that are called for only in a monograph.

The sense of proportion which rendered Baker so distinguished as a systematic writer made him equally effective as a teacher. He used the natural history of plants as a means of educating those on whom he inculcated the importance of botanical knowledge in everyday affairs. His style was lucid and concise, while he possessed the happy gift of ability to emphasise the salient features of his subject without neglecting its details.

Baker's published works ensure the perpetuation of his memory as the last of a singularly gifted circle of systematic botanists. While any of them survive, those who worked with or were taught by Baker will cherish the recollection of one of the kindest and best of men.

D. P.



Chas. Lapworth

CHARLES LAPWORTH, 1842–1920.

In the death of Charles Lapworth in his seventy-eighth year, the science of Geology mourns one who, in the amount and quality of research performed, by the current of fresh ideas with which he was inspired, and in the new direction imparted to the work of his pupils and contemporaries, stands out as one of the leading geologists of his time, worthy to rank with the foremost of the pioneers. When he began his work the glamour of the first fine flush of geological discovery had paled, and it seemed as though the boundaries of the science had been reached, the leading facts disclosed, the main principles laid down. Before his death, the science had been born again, and new discoveries showed that we were but on the threshold of a great development. The effect of his work was most marked in illuminating the structure of our own country and particularly the older part of it, but the work was so thoroughly done, and the principles involved so soundly established, that its reactions were felt in Scandinavia and Bohemia, in America and Australia, in the Dolomites and the Festoon Islands, while it even touched the ocean depths and the Antarctic continent.

His first series of researches brought about the realisation of his forecast that the history told by the Older Palæozoic rocks could be broken up into delicate time divisions comparable with those which heretofore had only proved possible of establishment in the Secondary and Tertiary rocks. This task was effected in the Southern Uplands of Scotland, where he happened to be residing. The rocks in this district are so highly disturbed and convoluted that they had defied the efforts of the best stratigraphers of the time to unravel them. Lapworth was at his best when grappling with a really difficult problem, and, as he saw the necessity for it, he sedulously cultivated those arts of his profession which he realised would alone enable him to solve it.

Large scale maps were essential—he would survey the ground and produce them; lithological variations were minute and obscure—he cultivated his eye and mind to detect and remember them; the geological mapping of these variations must be painstaking and thorough—even though such detail had never yet been attempted in a complicated district he would carry it to completion; distances were long and arduous—no exertion should be too great or hardship too severe; work on sections and maps must be delicate and minute—he would train his hand and eye as an artist; graptolites were the only fossils—he would learn all that was known about them and find out for himself what else there was to be learnt; search for fossils must be thorough and exhaustive, collection laborious and exact—he knew that this was vital and did not shrink from carrying it out.

So he worked for fourteen years; first at Galashiels, then in Roxburgh and Selkirk, next at Moffat, which he realised was the key area, and finally he put his deductions to the test in the baffling ground of Girvan and Ballantrae. He succeeded in proving that the Moffat Shales—a black, fine-grained, deep-water, deposit, some 300 feet thick—represented in full the thousands of feet of ashes, grits, greywackes, and slates of Upper Llandeilo, Bala, and Llandovery ages in Wales and England: That, in their thin beds, foot by foot and sometimes inch by inch, distinct graptolitic faunas were embedded, succeeding one another in an unvarying order, comparable in part at least, to their succession in much thicker masses elsewhere: That the shale bands themselves were the highest part of a series of rocks underlying the Silurian greywackes of the Uplands, brought to the surface in narrow anticlines, which were generally inverted and always complicated by faulting: That this region, instead of possessing a simple ascending sequence, as had been supposed, was really one of intense plication and convolution, so much disturbed that anticlines had been mistaken for synclines, to the effect that the apparent succession was upside down: And that there existed here mountain structure of the same type and order as was being worked out in the Alps.

In the Girvan region he found confirmation for all his conclusions, using the graptolites that he discovered here, too, as his clue, and mapping the difficult country as faithfully as before; and thus he set in order a group of rocks very much thicker than at Moffat, and containing faunas of trilobites and shells intercalated among his graptolite zones.

By this time he had worked out the life sequence of about half of the Lower Palæozoic rocks, dividing their history into a dated zonal calendar so soundly constructed that events all over the world have been found to fit into it. The same results could have been worked out years before, in half-a-dozen easier regions, where the sequence was laid out in simple order, had it but occurred to any observer that such minute work was either necessary or desirable, and had any geologist tested organism after organism until he hit upon those which best served his purpose. Lapworth effected it in a region where there was not a single straightforward section, among traps and pitfalls innumerable, and where the rocks were as often on their heads as on their heels. Well justified was the note of triumph in his words: "Zonal work is probably destined to effect in the history of geological research a revolution as great and an advance as rapid as those brought about by the use of the microscope in the history of biology."

He recognised the debt he owed to the graptolites for his success, and he was not the man to leave a debt unpaid. He made a careful study of all that was known, or to be known by his own researches, of their biological characters and geological distribution. He arranged his holidays so that he could collect them all over the country, examined those sent him from other areas, whether in Britain or Overseas, classified them afresh and described a host of new forms, and finally established some twenty zones in his own district and outside, which he found were not only of world-wide extent, but so exact that in several instances he was able to correct errors into which local observers in Wales and America had been led by delusive stratigraphy. His magnificent paper on the 'Distribution of the Rhabdophora' came like a revelation to his co-workers, so many unexpected results did it disclose, and so thoroughly had Lapworth realised his dream of zoning Lower Palæozoic rocks, not merely as satisfactorily as had been done for the Neozoic rocks, but, in truth, far more so. For these zones are more constant over wide areas than are those in newer rocks, the reason, worked out by Lapworth, being that the graptolites were pseudo-planktonic in habit; attaching themselves to seaweeds, they were drifted right over the seas, and became embedded in the fine sediments deposited in the ocean depths.

The influence of this research upon contemporary work is shown in the 'Monograph of British Graptolites' by Miss Elles and Miss Wood, written for the Palæontographical Society under Lapworth's editorship, in which the Lower Palæozoic zones are shown to have increased from 20 to 36, and the number of species dealt with from 284 to 372. The graptolites were also made to yield their contribution to the study of evolution; the minute variations exhibited by the species in ascending sequences, which in the first instance were observed for their geological bearing, being evidently directed towards improved conditions of life and promoting the survival of the fit.

Lapworth's third piece of work relates to the ancient rocks of the Midlands, beginning in 1881, the date of his appointment as Professor at Birmingham. The Ordovician rocks of Shropshire were studied in detail, and paralleled with those of Scotland and elsewhere. The Upper and Middle Cambrian rocks were brought into order, and to some extent zoned. Two distinct types of Lower Cambrian rocks were individualised in Shropshire and Warwickshire, fossiliferous rocks of this age being thus demonstrated to exist in England, and their chief fossil being reconstructed and described. The Welsh Lower and Middle Cambrian rocks were mapped at Harlech and compared with those of the Midlands, just as these in their turn were found comparable with those of the Highlands of Scotland. Deeper still, the rocks of the Longmynd, the oldest massive sediments yet known in the country, were for the first time mapped in detail, proved to be earlier in date than the Cambrian, and compared as to their higher members with the Torridon Sandstone of Scotland, and, as to their lower members, with the problematical rocks of Charnwood Forest in Leicestershire. The underlying rock of the Uriconian group was also mapped in Shropshire, and the occurrence of related rocks proved under the Cambrian of Worcestershire and Warwickshire.

It was only fitting that one so steeped in the facts should lay down the law as to the nomenclature of the Older Palæozoic rocks, and particularly that Middle Group, of which, in Scotland, England, Wales, and Ireland, he had become *facile princeps.* The work of the "old masters" had left this middle group in dire confusion. Lapworth cut the knot, and, impressed by the existence of three great and sub-equal faunas in the Older Palæozoic rocks, he erected his "Ordovician System" to rank with the Cambrian below and the Silurian above. Thus he earned the grateful thanks of geologists of his own and later times, for a work which could only have been done by one of his great knowledge, and which would never have been accepted but for the respect in which its author was held, and the knowledge, skill, and tact with which he stated his case.

The fourth piece of work, which will always be associated with Lapworth's name, was to overcome the great stratigraphical difficulties which had led to a mis-reading of the complicated sections of Lower Palæozoic and Eozoic rocks in the North-west Highlands of Scotland. Sir Jethro Teall, who (one of very few) had the advantage of seeing Lapworth's methods of work in this region, and who afterwards studied the petrology of the Highland rocks and took part in the publication of the 'Geological Survey Memoir,' has been so good as to furnish the following account of the Highland work:—

> I have been asked to say something about Lapworth's work in the north-west of Scotland, and I do so with great pleasure as it was my good fortune to spend a day or two with him in Eriboll in 1883. But before describing what was to me a memorable experience, it will be necessary to give a brief account of the situation as it stood when he first visited the district in 1882, and of his work in that year.
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> Having established the principles by which the complicated stratigraphy of the Southern Uplands could be interpreted, and applied those principles with brilliant success to the Moffat and Girvan areas, he felt free to turn his attention to the Durness-Eriboll region, where Lower Palæozoic rocks were also known to occur. In the fifties and sixties of last century, a keen controversy had arisen between Sir Roderic Murchison and Prof. Nicol of Aberdeen, concerning the relation of these fossiliferous rocks to the "Eastern Schists" of Sutherland and Ross. According to Murchison there was a gradual upward passage from fossiliferous strata to the crystalline schists, whereas Nicol maintained that the superposition had been brought about by faulting and that the highly metamorphosed "Eastern Schists" were older than the comparatively unaltered sediments—not younger as Murchison supposed.
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> This controversy had recently been revived by Hicks, Callaway, Bonney, and others, and although much new light had been thrown on the subject, the problem had not been definitely solved. The fact that previous observers had arrived at diverse conclusions, for even the opponents of the Murchisonian view were not agreed on all points, convinced Lapworth that some secret lay hidden in the district; and so, equipped with the six-inch Ordnance maps, with a knowledge of mountain structure derived partly from his own work in the Southern Uplands and partly from the work of Rogers in the Alleghanies, Heim in the Alps, and others, but above all with his own genius, skill, and enthusiasm, he set to work on his self-imposed task.

He began in 1882 at Durness where the evidence of the superposition of the Eastern Schists on the fossiliferous rocks is conclusive; but not finding, in that region, a clue to the solution of the difficulty he soon moved on to Eriboll, taking up his quarters on the east side of the loch at a shepherd's house, near Heilem Ferry. Here he found what he wanted; a succession of well stratified rocks divisible into zones suitable for mapping purposes and a district in which the rock-exposures were sufficiently numerous to enable the distribution of the different zones to be recorded on his maps. It must be remembered that the fundamental difference between the work of Lapworth and that of all previous observers is, that he attacked the problem in the only way that it could be satisfactorily dealt with; namely, by geologically surveying the district on maps sufficiently large in scale to bring out the extremely complicated structure. His main work was done on the six-inch maps, but in some areas this scale proved inadequate.

At the end of the short summer holiday he had surveyed a considerable area, extending from Whiten Head to Eriboll House, in sufficient detail to bring out the main structural features and to disprove the theory of the upward succession from the unmetamorphosed fossiliferous rocks to the crystalline schists.

On returning home he began a series of articles in the 'Geological Magazine' (March, 1883), entitled "The Secret of the Highlands," stating his general conclusions in the following words (p. 121):—

"I believe that we have in the so-called metamorphic Silurian region of the Highlands of Scotland a portion of an old mountain system, formed of a complex of rock formations of very different geological ages. These have been crushed and crumpled together by excessive lateral pressure, locally inverted, profoundly dislocated, and partially metamorphosed. This mountain range, or plexus of ranges, which must have been originally of the general type of those of the Alps or Alleghanies, is of such vast geological antiquity that all its superior portions have long since been removed by denudation, so that, as a general rule, only its interior and most complicated portions are preserved to us. In the area partly worked out by myself, the stratigraphical phenomena are identical in character with those developed by Rogers, Suess, Heim, and Brögger in extra-British mountain regions." This series of articles was never completed for reasons that will appear later.

In the following year (1883) he returned to his task, and my acquaintance with him arose in this way. In the early summer of that year the late Prof. J. F. Blake and I met Lapworth at Rhiconich, in Sutherland, by accident, and went on with him to Durness, where we found Peach and Horne, who were then beginning the official geological survey of the district. Lapworth very kindly invited Blake and myself to accompany him to Eriboll, an invitation which we gladly accepted. On the way he called our attention to the unconformable junction of the quartzite with the Lewisian gneiss, and to the lithological zones in the quartzite. This was necessary because only the upper part of the quartzite—the "pipe-rock" of Nicol—is exposed in Lapworth's type section at An-t-Sron, on the other side of the loch. Our first work in Eriboll was to examine this section. There he demonstrated the normal succession from the pipe-rock of the quartzite, through the "fucoid" beds and the salterella grit, to the limestone, directing our attention to the various zones—seven in all—which he found useful for mapping purposes, and had already described in his paper on 'The Secret of the Highlands.'

During the remainder of the time we were with him, about two days, he took us rapidly over the ground that he had surveyed in the previous year between Eriboll House and Whiten Head. First he proved to us that the zonal succession which he had established at An-t-Sron was repeated on the opposite or eastern side of the limestone basin, with steep dips and other complications that it would have been very difficult to unravel without a knowledge of the true sequence. At Arnaboll hill he showed us the Lewisian gneiss, resting on quartzite, called our attention to the abnormal aspect of both rocks, especially near the junction, and gave us his interpretation of the facts. The gneiss, he said, had been forced westward over the fossiliferous sediments on an overthrust-fault of low hade, and the abnormal aspect of the rocks seen here, and at many other places which we subsequently visited, was due to the fact that they had been crushed and in some cases rolled out by what he called the earth-mill, so as to be almost unrecognisable. He invited me to take specimens and examine them under the microscope, as he had done. Those specimens are now some of my most cherished possessions, for they introduced me to a new petrological world. He suggested that the term "mylonite" would be appropriate for rocks that represented the extreme phase of mechanical metamorphism, and this term, now well established, he finally adopted.

Leaving Arnaboll hill we worked northwards towards Whiten Head, and, to remove any doubt that might linger in our minds as to the identity of the "Upper Quartzite" of Murchison with the "Lower Quartzite," he placed me on the latter with instructions to walk along it, making sure that I never left it. He and Blake took up their positions on the "Upper Quartzite," and moved in a direction roughly parallel to me with the same care. Progress was slow, for in such a disturbed area it was necessary to examine every inch of the ground. Finally we met on quartzite, shook hands, and declared that beyond all shadow of doubt the "Upper Quartzite" was merely the "Lower Quartzite" brought up again by the disturbances of which we had already seen such striking evidence. As a further illustration of these disturbances he took me, after Blake had left us, to the shore north of Heilem, and showed me that the salterella grit had there been repeated many times on itself by clean-cut faults, a striking illustration of "*schuppen*" or imbricate structure, common in the Alps and elsewhere, but new to Britain.

The relation of the Eastern or "Moine Schists" to the complex of mechanically metamorphosed rocks which lie below them was then engaging much of his attention. He showed us that both groups had been, so to speak, alive with movement, and speculated as to the possibility of the latter representing a phase through which the former had passed, but his views on this great question were not at that time fully developed. The position he finally reached was summarised by him as follows in a communication read at the Geologists' Association on July 4, 1884, and subsequently published.* "I believe at present that the great area of metamorphic schists of Sutherland and the Central Highlands is, as a whole, neither Archæan nor Ordovician. The Sutherland Gneiss—Arnaboll—is Archæan. The Sutherland Schist has been manufactured since Silurian times. For all I know, there may be large areas (in the Central Highlands, etc.), composed wholly of Archæan (Laurentian) rocks, or of Cambrian or pre-Cambrian rocks. When the metamorphism of the Highland area began I think that it is impossible to say, and may be always impossible. One thing seems pretty clear to me—the so-called oldest beds of the Highland succession of the Schistose Series of the N.W. Highlands are the newest in point of time. The zone of intermixture and metamorphism (in Sutherland) travelled to west from east, and the last beds (schists) manufactured are those now in contact with the Assynt Series in Durness, Eriboll, and Assynt" (p. 441).

The subsequent work of the Geological Survey has not confirmed these views, except so far as the occurrence of Archæan (Laurentian) rocks among the Eastern Schists is concerned; but, as stated in the "Memoir on the Geological Structure of the North-west Highlands of Scotland" (p. 600): "The age and origin of the rocks that have been mapped as Moine schists is a complicated problem which has not been finally and definitely solved, but abundant evidence has been accumulated to show that under the influence of the post-Cambrian movements rocks of diverse age and origin have acquired a common type of structure, and that true crystalline schists have been simulated if not actually produced."

The short time we were with Lapworth supplied us with a continual succession of surprises. We did not know which to wonder at most, the remarkable stratigraphy of a type hitherto unknown to Britain, or the skill with which Lapworth was unravelling it. He had been full of energy and enthusiasm, relieved by occasional flashes of humour, during the whole of the time we were with him; but it was obvious that the strain, both mental and physical, was very great and before the end of the season he broke down under it. In his feverish dreams, as he lay ill in the shepherd's house below the Arnaboll ridge, he used to fancy that the gneiss was still moving westward on the overthrust fault which he had discovered and mapped, and that both he and the house would be crushed by the advancing mass. So ended Lapworth's work in the north-west of Scotland.

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* 'Proc. Geol. Assoc.,' vol. 8, pp. 438–442 (1885).

In 1883 the Geological Survey commenced their operations in the Durness–Eriboll region and on November 13, 1884, there appeared in 'Nature' a preliminary account of their work. It consisted of an Introduction by Dr. now Sir Archibald Geikie, in which he frankly abandoned the Murchisonian view, and a Report by Messrs. Peach and Horne which proved that the Surveyors had arrived at practically the same conclusions as Lapworth, both as regards the stratigraphy and the metamorphism. The following extract from a letter to me dated November 14, will show how he received the news that he had been forestalled as regards publication:—

"The matter has ended beautifully . . . I am too late with the publication of my results, but that is a matter of no consequence as the facts are out distinctly and clearly."

Referring to the communication to the Geologists' Association in his final paper "On the Close of the Highland Controversy,"* he wrote, on p. 98:—"It is not referred to in this place as establishing any selfish claim to priority, for the officers of the Survey reached their conclusions in complete ignorance of my results and from a totally different direction, while they have gone far beyond me in working out the details," and on p. 102, "The old subject of dispute has utterly disappeared and there is no longer any reasonable excuse for dissension. We have all been partly right and partly wrong. It is time for a hearty laugh all round, a time to shake hands and be friends."

It was Lapworth's intention to return to the district after the complete results of the work of the Geological Survey had been published, but the opportunity never occurred. This is to be regretted for there are certain statements in his communication to the Geologists' Association which require further elucidation. For example, he states that "in some cases the original dividing plane (either bedding plane or fault plane) of two successive rock-sheets has been twisted into the form of spirals, cornucopias, etc." It must be remembered that he possessed to a remarkable degree, the faculty of thinking in three dimensions. A few facts observed on the surface suggested to him a hypothesis as to the kind of deformation to which a rock-sheet had been subjected, or, in other words, as to its course underground and where it should reappear. The hypothesis was then tested by further observation and at once discarded if its predictions were not verified. He was constantly forming such hypotheses and discarding or modifying them until he found one that fitted all the facts obtainable. If he had carried out his intention of returning to Eriboll, the somewhat puzzling statement quoted above would doubtless have been expanded and illustrated.

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* 'Geol. Mag.,' December 3, vol. 11, p. 98 (1885).

In comparing the published work of Lapworth with that of the Geological Survey, one is struck by the fact that Lapworth approaches the subject from what may be called the "fold" point of view, the Survey from the "fault" point of view. Lapworth did not attach much importance to this difference, for in a letter to me dated September 21, 1885, he wrote:—"The Survey men have gone beyond me in boldly grasping the idea that the same result can be arrived at quite apart from following the theoretical stages downwards inch by inch and simply asserting that under pressure the rock snaps—like a sheet of ice—flakes or shears in parallel slates which slide over each other. In that I agree, though I reach it from an opposite direction. I hold that overfolds, sigmaclines, overfaults, thrustplanes, are *homologous* and pass insensibly one into the other; exactly as the American geologists believe that monoclines and ordinary faults are *homologous* and pass insensibly one into the other. As under certain conditions (excessive tension and torsion) no monoclines are formed but simply *faults*; so under certain opposite conditions (excessive pressure and torsion) no overfolds are formed but simply thrustplanes (my overfaults)."

J. J. H. T.

As the distribution of the humble graptolites—"outils qu'il a forgés lui-même et que d'autres eussent dédaignés"—had established world-wide time horizons, and had unravelled directly the mountain structures of the Uplands—and indirectly those of the Highlands—so were these last in their turn to be applied to the tectonics of the broader features of the earth-crust.

In this, as in his other work, while possessing deep and sympathetic knowledge of the researches of such geologists as Suess, Heim, Bertrand and Brögger, he held steadily to the views of the mechanics of the earth's crust to which his independent thought had led him. In his view the structure underlying rock complication was the "fold," Hogarth's "line of beauty and grace," sometimes tearing into faults or breaking down into cleavage, and of all dimensions from microscopic to mountainous. Lapworth, in his epoch-making address to Section C of the British Association at Edinburgh, and in later addresses, showed that the continents were but the crests and the oceans but the troughs of great earth-waves, with the septum between the two gentle and inactive, or else abrupt, advancing, and alive.

The greater continental crests are generally sagged downwards, and the oceanic troughs buckled upwards, at their centres. The "land hemisphere" of the world, with its central sag, the Atlantic, has its counterpart in the hemispheric Pacific depression, the one divided from the other by the greatest septal line of the globe, the "Pacific girdle of fire," "ablaze with volcanoes and creeping with earthquakes."

The "Challenger" had revealed for the first time the true or planetary contour of the earth crust, discovering a new world larger than that fraction of the globe hitherto known, the land part of it. It had depicted the plateaux on which the visible continents stand, separated by steep (septal) slopes from the deep ocean floors. Lapworth showed that the calculations based on these discoveries were consistent with and were explainable on his "fold theory." The area of ocean bed below the mean line of the septal slope is equal to the area of the earth-crust above it, while the bulk of ocean water below that level is equal to the crust material projecting above it.

Stretching from pole to pole, he states, we have three great crests, America, Eur-Africa, and Asia-Australia, with their three troughs the Pacific, Atlantic, and Indian Oceans. At right angles to them are the Arctic Ocean, the Mediterraneans, and the Southern Ocean, with their continental crests in the latitudes of North America, South America, and what, if the theory is sound, must be an Antarctic Continent. The interference of these two sets of master-folds accounts for the position of the great land-masses, their oblique coasts and their triangular terminations, and for the ocean deeps. Analogies to these may be detected in the moon and planets, and in the sun, while, in theory, they may possibly be found passing outwards and upwards, in the spirals of the nebulæ and in "that most glorious septum of all the visible creation the radiant ring of the Milky Way."

There has been no space to dwell on other sides of Lapworth's character or activities. His gift for teaching in the class-room and the field; his inspiring influence on his co-workers; his services to the State in relation to Geological Surveys and Coal Supplies; his bid for the freedom of research from the shackles of convention and authority; his genius for the grouping of facts, and the scientific use of his vivid imagination as a tool in his own research and a generous stimulant to investigation and discovery in others; his elaborate care that the results of clear thinking should be as clearly and logically expressed in his own writings; and his profound belief in the importance in education and in life of science generally, and his own science in particular, "not only the interpreter of Nature, but also the servant of Humanity."

Though his friends well know that his work, founded on the truest devotion to scientific principles and a passionate love of truth, can never die, they must henceforth miss the kindly and genial presence, the rich stores of many-sided wisdom and experience, the boundless energy and enthusiasm, the flashes of genius and inspiration, the transparently beautiful character of him who is no more.

> "His life was gentle; and the elements
> So mix'd in him, that Nature might stand up,
> And say to all the world, 'This was a man!'".

The portrait is from a painting by Mr. Bernard Munns and is reproduced with the kind permission of the Council of the University of Birmingham.

W. W. W.

Photo: Elliott & Fry.

Leonard Doncaster

LEONARD DONCASTER, 1877—1920.

THE death of Leonard Doncaster at the age of 42 has stopped a career of exceptional promise. He was a natural investigator, driven to research by the impulse of scientific curiosity, and his work will have a permanent place in the history of genetics. Born at Sheffield, December 31, 1877, the son of Samuel Doncaster, manufacturer, and his wife Emma Gertrude, whose maiden name was Barber, both members of the Society of Friends, he was one of the many naturalists produced by that body. Throughout his life the Quaker principles governed his development and bearing towards the world, an influence which naturally became marked during the period of the war. He was sent to the Friends' Public School, Leighton Park, Reading (1890—5), going thence to King's College, Cambridge (1896), where he took an open scholarship. Before coming up he spent six months at Heidelberg, and thus had the great advantage—to a biologist—of some knowledge of German from the start. In the Nat. Sci. Tripos, Part II, 1910, he took a First, with a mark of distinction in Zoology, rarely given.

Though a zoologist by formal choice, he might equally have been a botanist. From childhood there was never any doubt as to the leading purpose of Doncaster's life. The problems of biology were always in his thoughts, and the form in which they presented themselves was to him indifferent. As a young student he was already a competent field botanist and entomologist, with some knowledge also of the domesticated animals and plants, much of which he had acquired in his father's well-known and beautiful garden. But, though his range was wide, anything like vagueness or superficiality was quite alien to his composition. He liked knowledge hard and clear; and his weaknesses were not those of the omniscient or the expansive. Circumstances led him into academic zoology, but he never lost touch with these varied interests. Biology was probably to him, as to so many modern naturalists, rather a challenge than a source of contemplative enjoyment. A hint, which could be used in the attack, might come from anywhere.

After taking his degree he spent some time at the Naples Station (1901—2). He had there two objects in view, the first being to make a fresh investigation of the structure and development of *Sagitta*. In this animal there is an ovary and a testis on each side of the body; and since the two organs, male and female, of each side arise directly by division and differentiation of a single common mother-cell, there was a chance of seeing something exceptionally interesting in the cytological phenomena by which this process is accomplished. A useful paper on *Sagitta* was the outcome of this work, but cytologically the material proved intractable. His second purpose at Naples was to find out why such various and discordant results had been encountered by previous experimenters on the hybridization of Echinids—a problem which had naturally acquired fresh interest from the recent discovery of the Mendelian system of analysis. Up till that time the observation that a cross between two species did not give a uniform result was accepted without demur, but now such an occurrence was anomalous, and called for special consideration. A great deal still remains to be done in this vexed field. Doncaster, however, was successful in contributing one new fact, namely, that the seasonal changes observed by H. M. Vernon were in the main directly dependent on temperature. His experiments, which were very laborious, also demonstrated a curious series of individual differences in the degree of dominance—a phenomenon which should be examined further.

On his return to England he attached himself definitely to the group of studies now called genetics, and began a number of investigations, most of which involved experimental breeding. At various times he bred rats, cats, pigeons, besides gall-flies, moths, and other invertebrates. The early struggles of Mendelism to obtain a hearing were then in an acute stage, and though strongly inclined to caution, he knew enough of the general course of variation and heredity to be in no doubt as to the essential truth of the new doctrines. His adhesion certainly helped greatly in spreading confidence among his contemporaries. His first paper on a definitely Mendelian problem was a note on the Tortoise-shell Cat, in which he showed that the well-known rarity of tortoise-shell tom-cats is an expression of the fact that whereas the cross between orange and black produces female tortoise-shell, the corresponding male form is orange. About this time he began an investigation into the life-history of the saw-fly, *Nematus ribesii*, and of the gall-fly, *Neuroterus lenticularis.* The latter subject he pursued with intervals for more than ten years. The purpose was to discover the mechanism by which sex was determined in these species. The fertilized eggs of *Neuroterus* give rise to females only, as do those of *Phylloxera* and some other Hemiptera. These females, unfertilised, produce other females of which (again without fertilisation) some produce males, and others females, not a mixture—a condition now known to be parallelled by various insects. The cytological distinction, if there is one, between these two types of females, is still undiscovered. Doncaster's work on these and similar types, and especially the clear exposition which he gave of their intricate polymorphism, contributed much towards the comprehensive codification by which a mass of apparently contradictory records as to the parthenogenetic or agamic and sexual forms of Hymenoptera and Hemiptera has been reduced to order.

He will be, however, best remembered for his experiments on the inheritance of sex in *Abraxas grossulariata,* the Currant Moth. From the Rev. G. H. Raynor, who had long been a fancier of this species, Doncaster learnt that the variety *lacticolor,* distinguished from the common form by a great reduction in the amount of black, was known only in the female. At that time no example of what is now called "sex-linked" inheritance amenable to experiment had been studied. He at once saw the extraordinary importance of the subject, and, as the result of correspondence with Mr. Raynor, matings were arranged and a critical investigation of the case was begun. It was soon found that by mating *lacticolor* females with F_1 males (from *lacticolor* female × *grossulariata* male), the missing *lacticolor* male could be produced, and the various possibilities tested. The facts proved that the system of inheritance is exactly the converse of that previously known to be followed by colour-blindness and certain other conditions in man. Whereas the linkage of colour-blindness is with maleness, that of *lacticolor* is with femaleness. The first clear proof, carried out by the method of experimental breeding, that sex is determined in the gametes, was then made. Incidentally, another very curious corollary followed, namely, that ordinary wild *grossulariata* females are actually heterozygous for *lacticolor*, though that variety is seen very rarely in nature. But the cytological proof that in certain Hemiptera sex is determined by the gametes of the male had recently been obtained by E. B. Wilson, and this great discovery had naturally impressed Doncaster. Since the spermatozoa of these Hemiptera were visibly dimorphic in respect of sex, and the eggs of *Abraxas* were no less demonstrably proved by breeding methods to be dimorphic, he doubtless felt that this dimorphism must be a condition generally obtaining among the germ-cells of *both* sexes, and he therefore devised a scheme of sex-determination (also proposed independently by Wilson) representing both possibilities. This compromise involved the conception that both sexes should be heterozygous in sex and the supposition that dominance attached to the gamete received from the female. Subsequently, he accepted the emendation by which the female only of *Abraxas* is regarded as heterozygous, as the male is in the Hemiptera and Diptera, the other sex in each case being taken simply to be homozygous, paradoxical though that conclusion is.

The next step was to look for a cytological proof of the dimorphism of the eggs, but this was not to be had. The diploid number was large, 56, and was only established with difficulty. But in the course of this further work a remarkable new phenomenon was encountered—that a certain strain of *A. grossulariata* had two kinds of females, of which one produced females almost exclusively, the other giving the usual mixture of sexes. Entomologists have met with great departures from the normal sex-ratios in Lepidoptera, but none had ever been investigated systematically.

Doncaster was still engaged on this inquiry when his final illness supervened. He found evidence of cytological distinctions between the two kinds of females, the female-producers having 55 chromosomes instead of the normal 56. He attempted an interpretation on the lines followed by Bridges in his paper on "non-disjunction" in *Drosophila*, but, as Doncaster pointed out, the suggestion was not consistently applicable, and much remained to be done.

In these latter years some further progress was made with the old problem of the tortoise-shell cat, which he had continuously kept in mind since the early days of Mendelism. As mentioned above, he had shown that, as a rule, the heterozygous combination of orange with black is tortoise in the female but orange in the male, a distinction which has not yet been factorially represented. But very rarely a male is produced having the characteristic tortoise-shell distribution of colour. From extensive inquiries among breeders, and from some direct observations of his own, Doncaster came on the interesting and suggestive fact that these rare tortoise-shell tom-cats are almost if not quite always *sterile*.

In his last paper of all, he put forward the original but by no means extravagant notion that perhaps the tortoise-shell tom is a free-martin, owing its peculiarity to intra-uterine influence of other female embryos. This conjecture was made in consequence of Lillie's surprising discovery as to the nature of the bovine free-martin. Most of these subjects were discussed in his useful text-book, 'The Determination of Sex,' 1914, but of course in the six years that he lived after that publication much progress was made. In regard to the chromosome hypothesis his views were at that time in a transitional stage. It may be noticed, for instance, that in discussing the descent of colour-blindness he does not develop the cytological argument. Normal colour-vision is represented as depending on the presence in the male of a single factor N, the loss of which produces in him colour-blindness. The normal female is homozygous in N, the transmitting female, whose sight is normal, being Nn, like the normal man. This was the notation which he had proposed in 1911, and it has been adopted as an improvement on all previous suggestions. But in man the normal distribution of the sex-factor must be the same as that of N. The inference that the two factors, the one for colour-vision, the other for sex, are transmitted in collocation beomes inevitable; though till man is proved to have a distinct sex-chromosome, the nature of the collocation might be left to the imagination. Throughout the book also he never loses sight of the somewhat ill-defined though unquestionable evidence as to the possible modification of sex-ratios by influences of some different order, a circumstance which has hitherto not been reconciled with cytological appearances.

But of the various modes of attack on genetical problems his mind turned perhaps more naturally to cytology than to any other. Laboratory methods were congenial to him. He came to regard the empirical results of experimental breeding more and more as a stimulus to microscopical search for some visible basis of difference to which genetical diversities could be referred. The element of apparent fundamentality which he found in cytology very strongly appealed to Doncaster's analytical mind, and he was therefore from the first greatly attracted by the theory of linkage propounded by Morgan. In the clear and excellent 'Cytology,' which he published shortly before his death, he declares himself an adherent, a judgment which, from a student so slow to form decisions, has special value. At a moment when the claims of cytology are acquiring such prominence, his loss will be severely felt, for we have in this country no one who combines, as he did, personal experience both in all the branches of genetics and in cytological technique.

He was an exceptionally clear-headed thinker and speaker, full of enthusiasm and faith in the value of his work, and therefore an admirable teacher. From 1906–10 he held a zoological post at Birmingham University, being for the latter part of his tenure of that appointment Lecturer in Heredity and Variation. In 1908 he married Dora, daughter of Walter Priestman, of Birmingham. Returning to Cambridge, he served the University in various capacities, especially as Superintendent of the Museum of Zoology (1910–14), and Lecturer in Zoology (1911–17). When, in 1919, Professor Herdman resigned the Derby Professorship of Zoology in Liverpool University, Doncaster was appointed his successor. To geneticists this appointment was a source of great satisfaction. It seemed that a fresh centre for the development of these interests was assured. He began the work of his new Chair with all his zeal and devotion. But within a year he was struck down with malignant disease, and died May 28, 1920.

In a notice of his "fine young colleague" which appeared in the 'Liverpool Daily Post' of May 29, Professor Herdman wrote :—"Doncaster was a splendid lecturer, and an investigator of the first rank. But what struck one most, beyond these high qualities, was his absolute right-mindedness, his serious conscientiousness, his evident determination to do what he felt to be right under all circumstances. We have all alike been impressed by the care and trouble that he took, by his sound judgment, and the weight of his considered opinion."

Personally, Doncaster was slight in build, and in temperament intellectual, highly strung and somewhat anxious—a combination not rare among the advancers of knowledge. His mind was always working, and he felt and thought of everything with concentration and intensity. The years of the war were, I believe, to him a period more horrible than to most thoughtful men. He held strongly the Friends' attitude of the unlawfulness of war, but feeling that alternative service was a duty, he gave up his researches and qualified as a bacteriologist, working first in one of the Cambridge military hospitals, and afterwards in the Friends' Ambulance Unit at Dunkirk.

Holding these reservations from the common ways of men, he never joined quite easily in ordinary society. That religion was a prominent element in his nature was well known to his scientific friends, but it made no obvious difference in his demeanour towards us. This pre-occupation, latterly, came nearer to the surface, and in Cambridge he occasionally delivered religious addresses, it is reported, with distinction. At Dunkirk also he took part in the Sunday services of the Friends.

In several ways his work received outward recognition. He was Mackinnon Research Student of the Royal Society (1904–5). He was awarded the Walsingham Medal in Cambridge, and the Trail Medal of the Linnean Society.

In 1910 he was elected to a Fellowship at King's College, Cambridge, and in 1915 he became a Fellow of the Royal Society.

He leaves one son and two daughters.

W. B.

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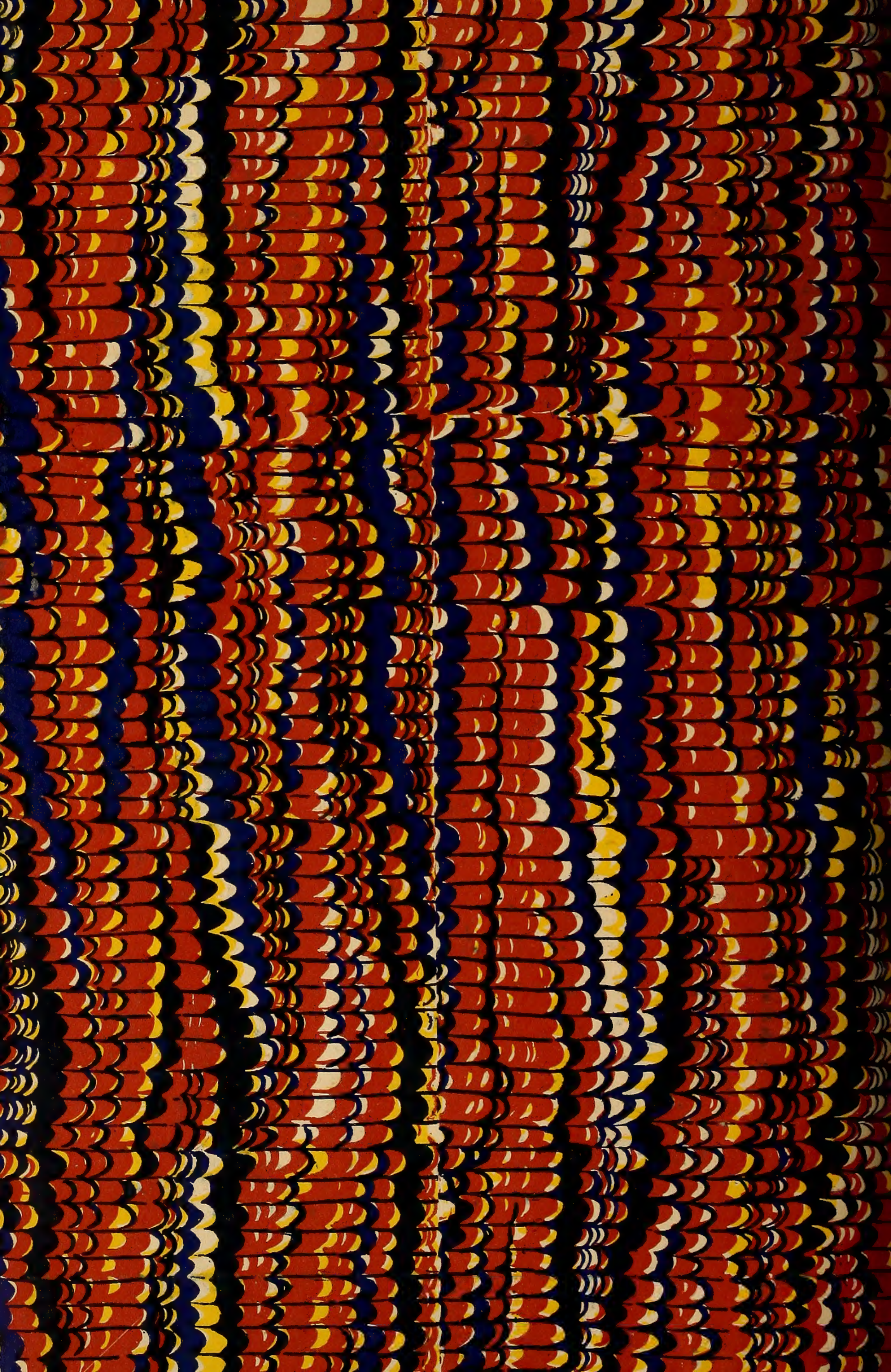

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